MINUTES OF THE MEETING OF
THE S-9 TECHNICAL ADVISORY COMMITTEE
FOR
PLANT GENETICS RESOURCES CONSERVATION AND UTILIZATION
S-9 REGIONAL PROJECT

Cooperative among:

THE STATE AGRICULTURAL EXPERIMENT STATIONS
OF THE SOUTHERN REGION

the
AGRICULTURAL RESEARCH SERVICE

the
COOPERATIVE STATE RESEARCH, EDUCATION, AND
EXTENSION SERVICE

and the
NATURAL RESOURCES CONSERVATION SERVICE

of the
UNITED STATES DEPARTMENT OF AGRICULTURE

JUNE 8, 1999

GEORGIA AGRICULTURAL EXPERIMENT STATION
GRIFFIN, GEORGIA

SUBMITTED BY

DON LABONTE, CHAIRMAN

PARTICIPANTS IN THE MEETING INFORMATION:

TAC Members:

Don LaBonte, Chair	Louisiana State University
Bill Branch	University of Georgia
Bryan Brunner	University of Puerto Rico
Ken Quesenberry	University of Florida
Bill Rhodes	Clemson University
Tom Stalker	North Carolina State University
C. M. Taliaferro	Oklahoma State University
Clarence Watson	Mississippi State University
Dennis West	University of Tennessee
Jerry Arkin,	Administrative Advisor to S9 Technical Committee, Griffin, GA

PGRCU Staff:

Robert Lynch, Acting Research Leader
Robin Dean, Bioinfomatics
Graves Gillaspie, Research Plant Pathologist and Vigna Curator
Rella Harrison, Administrative Secretary
Donnie Hice, Assistant Grass Curator
Mark Hopkins, Support Scientist, Molecular Genetics
Robert Jarret, Research Horticulturist and Vegetable Curator
Gil Lovell, Agronomist and Grass Curator
Brad Morris, Agronomist and Annual Clovers and Special Purpose Legumes Curator
Roy Pittman, Agronomist and Peanut Curator
Merrelyn Spinks, Data Base Manager and GRIN Coordinator
James Strickland, Farm Manager

Other Attendees:

Ann Blount, University of Florida
Peter Bretting, National Program Leader, Plant Germplasm
Christina Walters, Research Leader, National Seed Storage Lab, Ft. Collins, CO
Mimi Williams, USDA, ARS, Brooksville, FL

ADOPTED AGENDA:

8:00 Call to order and opening remarks
Jerry Arkin, Bob Lynch

8:15 Status of the PGRCU (S-9) collection & Seed Storage/Database Management
Merrelyn Spinks

8:30 Presentations by staff: (~25 minutes for each person. This includes an 8-10 minute discussion period for each person)
Bob Jarret
Roy Pittman

9:50 *BREAK* (10 minutes)

10:00 Presentations continued
Brad Morris
Gil Lovell
Graves Gillaspie
Mark Hopkins (15 minutes total)
Merrelyn Spinks (15 minutes total)

11:30-12:30 *LUNCH* (sandwiches brought in)

12:30 Christina Walters (NSSL)
Peter Bretting (NPL)
Budget picture
Bob Lynch.

1:30 General open discussion

2:15 Break

2:30 TAC committee representatives meet privately to formulate report. Final version is sent by mail to S-9 unit.

4:00 Meeting for TAC committee representatives ends

SUMMARY OF THE DISCUSSIONS:

- Jerry Arkin noted that the AES Directors for S9 had granted funding for salary increases of the S9 staff plus an increase in funding of $20,000 for the next fiscal year. However, ARS has not increased the budget for the unit in 10 years. The needs for the PGRCU include an increase in the ARS budget plus funding for salary increases and a new Research Leader who has knowledge of germplasm, classical genetics/ molecular genetics, and administration. Ken Quesenberry asked whether the new RL position would be category 1 or 4 - Cat. 1.

- Merrelyn Spinks in her overview of the S9 germplasm collection pointed out that the unit will have been in business for 50 years in November 1999. (Appendix 1)

- Robert Jarret discussed the status of the various vegetable collections and of his research in molecular genetics. Among the needs that he listed for curation were additional pollination cages, more irrigated land, and additional funding. He also needs funding for sequencing and fragment analysis and additional operating funds for research. Discussion involved such things as the problems with intellectual property rights and the hindrance in acquiring germplasm. Bob Jarret also stated that the somatic mutation work in sweetpotatoes had only gone far enough to show that transposable elements are at work. Peter Bretting pointed out that microarray analysis and HPLC should be done in cooperation with those currently working in these areas and that the analytical problems with data from microarray analysis are massive. (Appendix 2)

- Roy Pittman discussed the status of the peanut germplasm collection and stated that he needed more cages for controlled pollination of wild peanuts with honey bees and an increase site in Florida. Discussions mentioned that many of the wild peanuts needed to be caged to protect against cross-pollination and needed bees in the cages to trip the flowers properly. Dr. Simpson still has most of the wild peanut lines at Stephenville and needs to get the items collected in Brazil into the collection in the US. Tom Stalker stated that we need to get photos or herbarium sets for the wild peanuts since it will be necessary for taxonomic purposes after Simpson retires. Getting additional items from the ICRISAT collection faces problems with transfer agreements. It was noted that cooperative regeneration of peanuts in the last couple of years was funded by the cooperators. Molecular genetic work is currently being funded by the $50,000 from S9 (which covers the salary and some operating funds for a research coordinator) and a small grant from the Georgia Peanut Commission for $4,000. (Appendix 3)

- Brad Morris discussed the status of the special purpose legumes and clover collections. He stated that winged bean is a problem for regeneration and that most of the guar collection is located at NSSL. These collections need more observation data other than phenotypic characteristics. Brad discussed potential impact several legume species may have in the industrial and pharmaceutical sectors. In addition, Brad discussed the use of AFLP technology in conjunction with morphological data to adequately assess genetic redundancy among a large subclover collection. Peter Bretting asked if there had been any attempts at CRADA support. This has not been attempted, and attempts at cooperation have failed because of the lack of funding. (Appendix 4)

- Gil Lovell summarized the grass collections with main emphasis on the warm season grass collection in which regeneration is a significant problem. Gil felt that the needs involved finding a new curator for the sorghum collection and a new person to handle the regeneration work of sorghum and pearl millet at St. Croix, more funding for Hibiscus regeneration, funding for support for regeneration in field services, and funding for viability testing. Discussion involved the portion of the sorghum collection that has been inactive and whether these should be stored at -20C. It would be costly to rebag in sealed envelopes, but some contended that it would be cheaper to rebag and freeze than to have the constant regeneration need. To the question of whether regeneration needs were mostly because of viability problems or decreased quantities of seeds Gil answered that we do not have enough viability data to make decisions on this basis. A proposal of putting the inactive sorghums lines into the freezer without rebagging was also discussed. Dr. Bretting said he would look into regeneration at Puerto Rico for items that are photoperiod-sensitive and are not susceptible to ergot. (Appendix 5)

- Graves Gillaspie discussed the Vigna collection and the pathology research. Discussion involved mostly the peanut stripe virus testing. It was pointed out by several TAC members that the S9 Committee and the peanut CGC had repeatedly recommended that no testing for the virus was needed for germplasm already in the US, but that all new introductions as well as seeds being sent to foreign countries requiring testing should continue to be tested as needed. Dr. Lynch indicated that we would follow the recommendation now since no economic loss has been observed. No comments to the contrary were expressed by Dr. Bretting. Needs include funding for a molecular genetic approach with SSR markers to study duplicity and genetic diversity in Vigna, a site for regeneration of photoperiod-sensitive Vignas (Puerto Rico tops the list), and funding for viability studies. (Appendix 6)

- Mark Hopkins presented a summary of the results from the molecular genetics group. The paper in the August issue of Crop Science details the use of markers to determine duplicity in sorghum cv Orange accessions. The seven SSR markers separated a number of groups of peanuts with only 2 of 19 that could not be separated. Thus far, seven good SSR markers have been discovered for *Paspalum*. Unit needs additional memory for handling the database that will be generated in the near future. (Appendix 7)

- Merrelyn Spinks gave a summary on the use of the newly established S9 web site. This web site can be reached by going to Repository Home Pages on the GRIN home page, click on Plant Genetic Resources Unit, then click on S-9 Regional Project. This site has annual reports, meeting minutes, etc. (http://www.ars-grin.gov/ars/SoAtlantic/Griffin/pgrcu/s9.html)

- Christina Walters (the new Research Leader at NSSL) reported on the results and goals of their unit. In summary these goals are 1) to increase longevity of samples in storage, 2) to increase the number of plant species that can be stored, 3) to increase the ability to preserve elite genetic combinations, and 4) to increase the efficiency of monitoring viability. She extended an offer of cooperation of her unit on any seed storage problems. (Appendix 8)

- Peter Bretting discussed ARS budget situations in germplasm. Increases are forthcoming on ornamental germplasm research, but spending caps of several years ago are hindering increases in other areas. The American Seed Trade Association has lobbied congress to double the budget for germplasm in the next few years. Dr. Bretting said that there is some hope that Griffin might get some additional funding by FY 2001.

- Robert Lynch reported on the status of the current budget situation for PGRCU. In summary, things are only getting worse and there will not be enough for operating funds in FY 2000 unless some changes occur. There was interest by the TAC on how the committee might help the Unit do things better so that funds could be made available. (Appendix 9)

-Information Update from CSREES Plant and Animal Systems (Appendix 10)

-Status of *Citrullus* Germplasm Collection-Final Report to the Watermelon Research and Development Working Group (Appendix 11)

NEXT MEETING INFORMATION:

The idea of holding the S9 meetings every other year was discussed, but this was tabled for another year since the feeling was that the new Research Leader would be on board by June 2000, and the TAC would need to meet then to meet this person. Therefore, the next meeting will be held in Griffin in June 2000, and the new S9 Technical Committee Chair will be Dr. Coffey of the University of Tennessee.

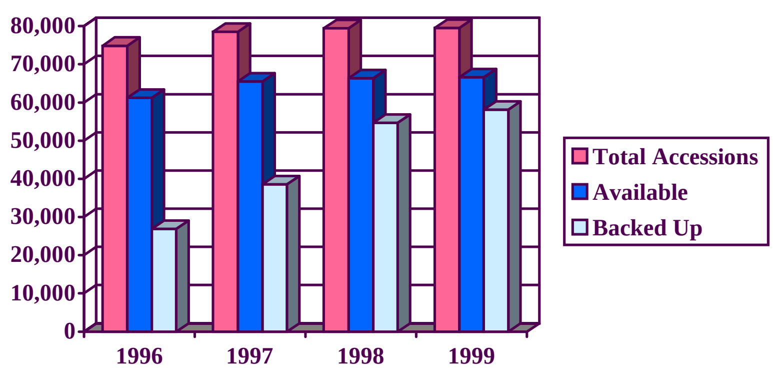
Appendix 1

STATUS OF THE
PGRCU (S-9)COLLECTION

Genetic Diversity of the Collection

- 79,502 Accessions (107,353 Inventory Samples)
- 264 Genera
- 1,491 Species
- 181 Countries

Four Year Comparison



Collection Status

- Core Collections
  - 4,725 accessions
  - 7 crops
- NSSL Only
  - 18,342 accessions
- Observation Data
  - 771,770 records

Service Units

Applied Genetic Analysis Lab
Field Services
Jim Strickland, Farm Manager
Richard Payne, Griffin
Jim Leaptrot, Byron
Amos Mack, Byron
Janet New, Byron
Pathology Services
Seed Storage / Database Management
Lee Ann Chalkley, Seed Storage Manager
Tiffany Bethune
Verlene Byous
Sylvia Jones
Lebus Kilgore
Merrelyn Spinks, GRIN / Computer Support

Seed Storage / Database Management

Accomplishments

1998 Orders

- Orders
  - Backup 43
  - Distribution 549
  - Information 90
  - Observation 21
  - Replenishment 70
  - Transfer 1

- Total Orders 774

- Order Items
  - Backup 7,217
  - Distribution 12,817
  - Information 3
  - Observation 2,451
  - Replenishment 3,418
  - Transfer 4

- Total Items 25,910

Other Seed Storage Activities

- Backup
  - 3,496 accessions

- Cleaning / Counts / Weights
  - 92,690 inventory samples counted
  - 14,663 remaining

- Freezer Storage
  - Original Samples
  - Other Crops
- Training
  - Orders
  - Observation Data
  - Fieldbooks

Bar Coding

- Crops not bar coded
  - Part of Sorghum
  - Peppers
  - Grasses
- Shipping system
  - Scan from storage bag label
  - Enter Inventory ID from keyboard
  - Off-line database from GRIN

Observation Data

- Data Loading
  - 379,450 records added
  - Sorghum CGC provided $10k
    - 334,130 records added
  - Clover/Legumes (5,236)
  - Grasses/Miscellaneous Crops (433)
  - Peanuts (29,141)
  - Sweetpotato (18,760)
  - Vegetable Crops (77,847)
  - Vigna (39,001)

Future Objectives - Crops to be Processed

- New to Collection
  - UCR Cowpeas
    - 162 accessions
  - Pearl Millet
    - St. Croix Quarantine increase
    - About 600 total
- Regenerations
  - 1998 Peanuts
  - Cowpea St. Croix increase
  - *Hibiscus* increase from Mexico
- Observation Data
- Imaging
  - Sorghum
  - Other crops:
    - peanuts
    - cowpeas
    - vegetable crops

Web Site

- PGRCU Home
- S-9 Project
- Enhancements
  - Scan S-9 RTAC Annual Minutes
  - Scan S-9 Annual Reports

Appendix 2

Vegetable Crops / Sweetpotato

Staff

Robert L. Jarret, Research Horticulturist & Curator
Research Coordinator – Vacant
Janice Smith, Curatorial Support

Crop	Total accessions	Number available (%)
Citrullus	1,643	85
Cucurbits	1,334	38
Eggplant	938	95
Gourds	464	53
Ipomoea	1,072	70
Luffa	159	80
Okra	3,060	50
Pepper	3,876	96

Crop	Total accessions	Core
Citrullus	1,643	N
Cucurbits	1,334	N
Eggplant	938	Y
Gourds	464	N
Ipomoea	1,072	N
Luffa	159	Y
Okra	3,060	N
Pepper	3,876	N

Crop	Total accessions	Backed up (%)
Citrullus	1,643	91
Cucurbits	1,334	63
Eggplant	938	98
Gourds	464	66
Ipomoea	1,072	70
Luffa	159	81
Okra	3,060	62
Pepper	3,876	54

Crop	Total accessions	No. NSSL only
Citrullus	1,643	332
Cucurbits	1,334	49
Eggplant	938	42
Gourds	464	12
Ipomoea	1,072	34
Luffa	159	1
Okra	3,060	41
Pepper	3,876	177
Total		688

Crop	Total accessions	Ob. Data loaded
Citrullus	1,643	11,137
Cucurbits	1,334	1,425
Eggplant	938	8,854
Gourds	464	782
Ipomoea	1,072	28,318
Luffa	159	193
Okra	3,060	5,534
Pepper	3,876	79,210

Curatorial Accomplishments:

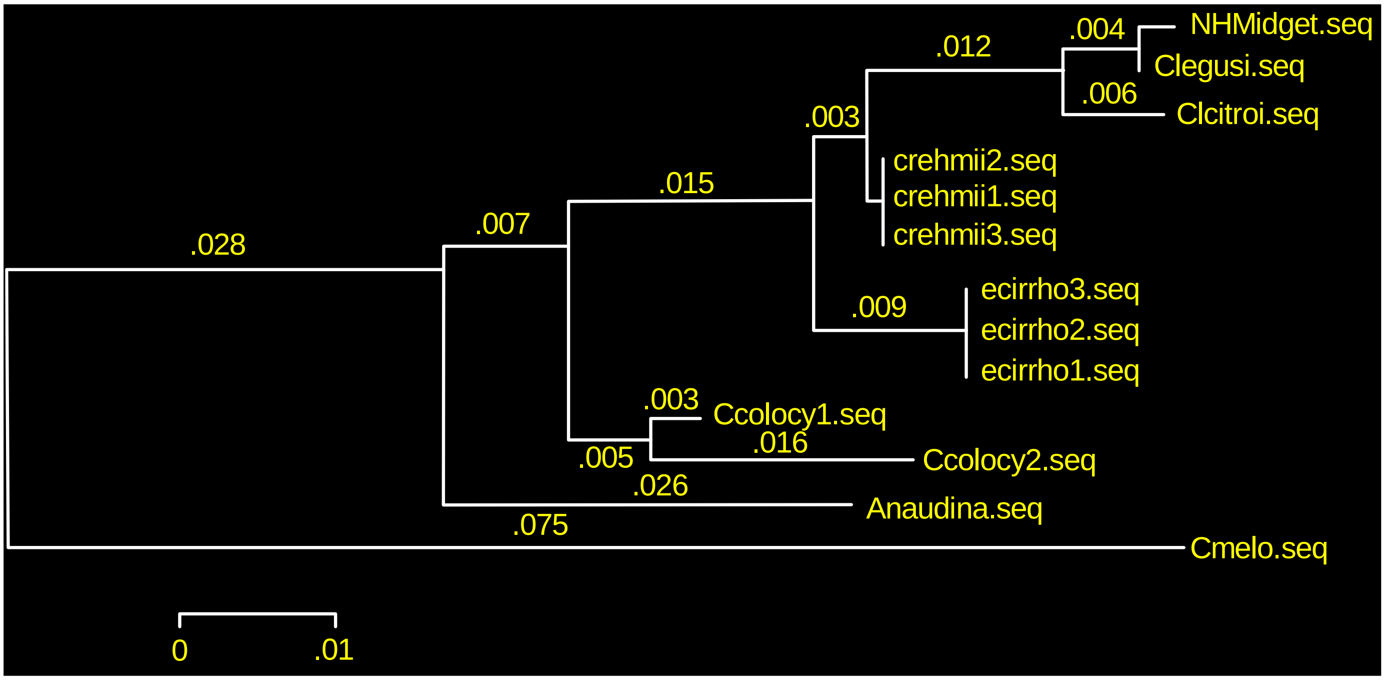
- *Citrullus* regeneration/characterization
- *Citrullus* spp. acquisition/regeneration
- *Capsicum baccatum* photo-documentation
- *Capsicum* spp. acquisition/regeneration
- *Capsicum* sp. plant exploration proposal
- Okra core collection regeneration
- *Solanum melongena* photo-documentation
- *Solanum spp*. plant exploration proposal
- *Cucurbita* spp. regeneration
- *Ipomoea batatas* regeneration/distribution

Research Accomplishments:

- Characterization and placement of *Citrullus rehmii* and *C. ecirrhosus*
- AFLP analysis for prioritization of *C. moschata* regeneration
- Continued development of molecular markers for use with vegetable crops
- Analysis of somatic mutations in sweetpotato

Curatorial Needs:

- Cages for increase of *Capsicum* and *Solanum*
- Additional drip-irrigated field space to permit crop rotation
- $$ Support for regeneration of *Cucurbita moschata*
- Modification of seed storage conditions?



Research Needs:

- Technical support for sequencing and fragment analysis
- Operating funds for same
- Microarray scanner?
- HPLC for germplasm evaluation?

Appendix 3

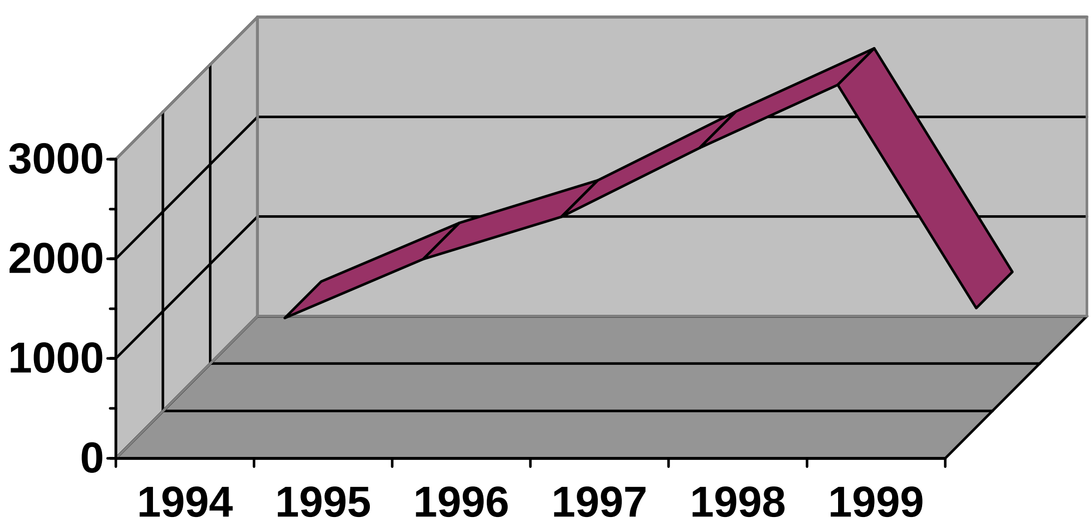
Arachis (Peanut) Project

Personnel

Roy Pittman, Agronomist & Curator
- Maintenance and Regeneration
  - Chris Jones, Cultivated Peanut
  - Stephanie Dunn, Wild Peanuts
- Genetic Analysis
  - Melanie Newman, Molecular Characterization

Increases for Cultivated Peanuts

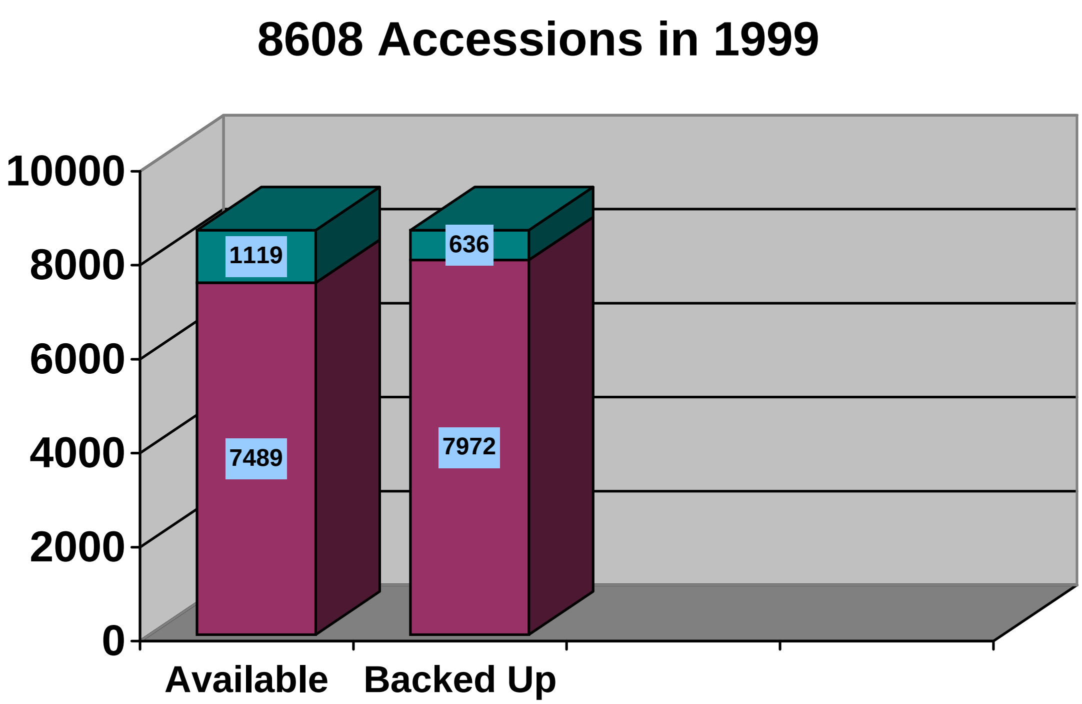
(1994 - 1999)



Cultivated Peanuts by Botanical Grouping

Arachis hypogaea	6639
Arachis hypogaea var. hypogaea	542
Arachis hypogaea var. hirsuta	31
Arachis hypogaea var. aequatoriana	62
Arachis hypogaea var. peruviana	22
Arachis hypogaea var. vulgaris	124
Arachis hypogaea var. fastigata	13

Status of Cultivated Germplasm



Cooperators (1)

Regenerations (1994-1999) by PCGC members

- Gorbet (FL)
- Holbrook (GA)
- Isleib (NC)
- Kirby (OK)
- Moore (GA)

Cooperators (2)

Research

- Holbrook B New Core Collection Members
- Peanut CRSP (Bolivia)
  - Dan Gorbet (FL)
  - David Zimet (FL)
  - Jim Todd (GA)
  - Albert Culbreath (GA)
  - Roy Pittman (USDA)

Cooperators (3)

Germplasm Evaluation

- Jim Todd (GA)
- Dan Gorbet (FL)
- Daryl Baker (NM)

Molecular

- Gary Kochert (GA)

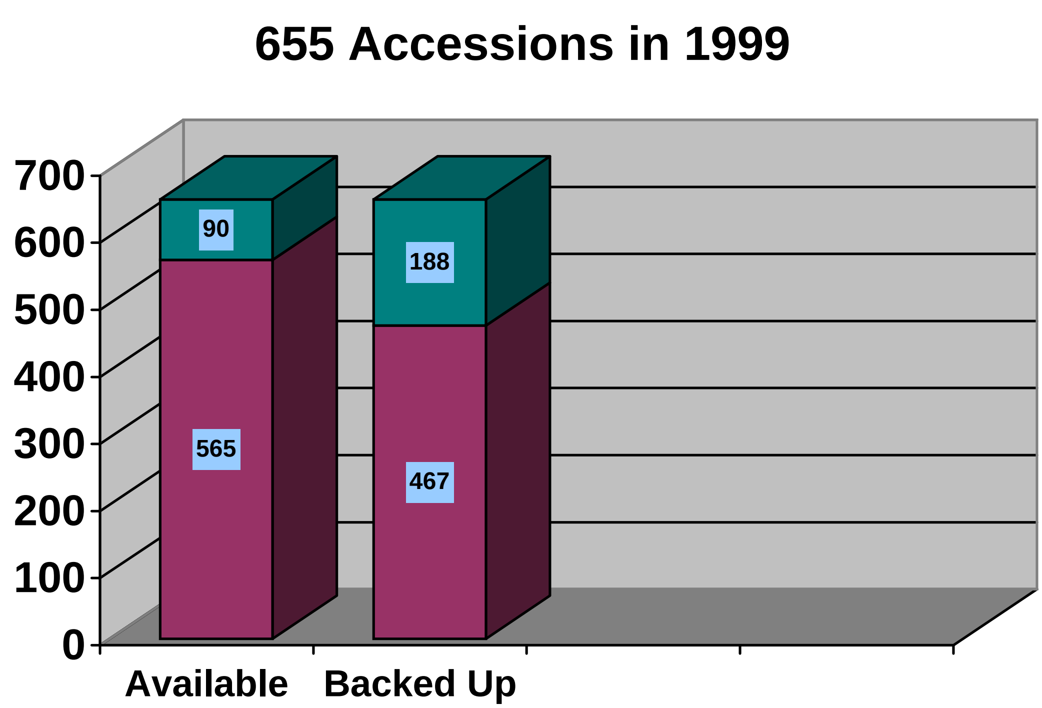
Wild Peanuts

Sixty-eight species are possible at present

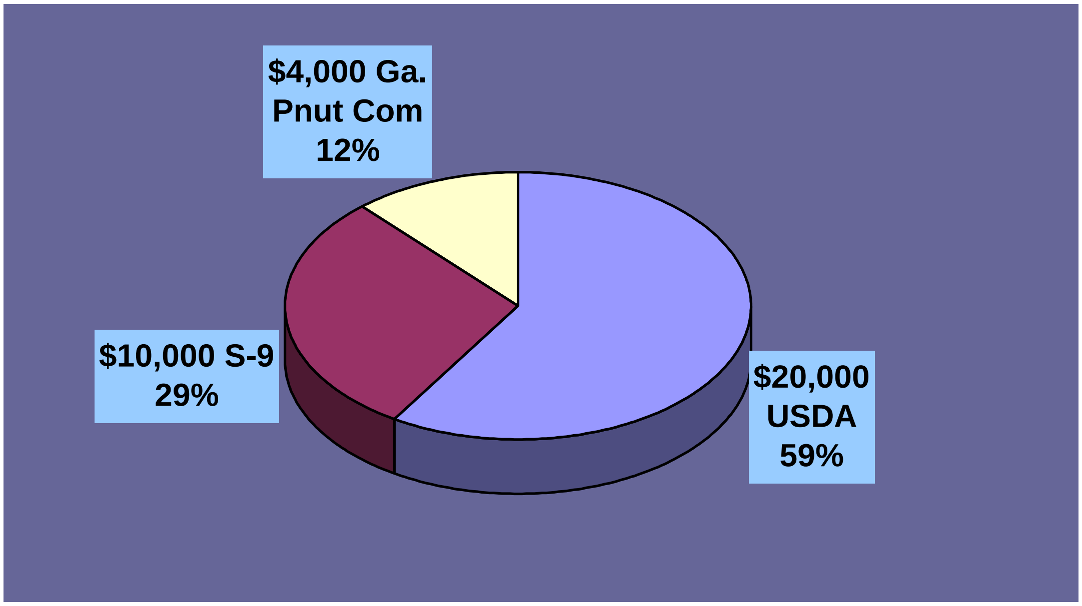
Not present in collection:

Arachis brevipetiolata
Arachis herzogii
Arachis lignosa
Arachis marginata
Arachis martii
Arachis trinitensis

Status of Wild Germplasm



1999 Peanut Budget



Molecular Research

Current

SSR=s

Discussion by Mark Hopkins

Possible Considerations
EST and/or SSR=s
Disease and Pest resistance (Ga & Fl)
OL trait (Fl)

Evaluation of core collection for allergens I, II, & III

Mapping population

Documentation of Germplasm

Digital Images
- Plant
- Pod
- Seed

Research or Curatorial Needs

New Priority: Wild Peanut Germplasm

- Controlled pollinations with bees
- Possible increase site in Florida
- Technical support at increase site
- Back up of wilds at NSSL

Appendix 4

CLOVER AND SPECIAL-PURPOSE LEGUMES

Curation/Research Activities

Brad Morris, Agronomist & Curator
Martis Watts, Curatorial/Research Support

5,450 accessions of approximately 424 horticultural, agronomic, ornamental, medicinal, and industrial species

Curation Objectives

Clover & Special Purpose Legume Availability

Crop	Total Accessions	Number Available
Guar	413	401
SP Legumes	2,906	2,223
Trifolium	1,965	1,349
Winged Bean	166	8

Clover & Special Purpose Legume Backed-Up

Crop	Total Accessions	Backed-Up
Guar	413	406
SP Legumes	2,906	2,299
Trifolium	1,965	1,428
Winged Bean	166	18

Clover & Special Purpose Legume Core

Crop	Total Accessions	Core
Guar	413	
SP Legumes	2,906	
Trifolium	1,965	95
Winged Bean	166	

Annual Clover & Special Purpose Legume at NSSL Only

Crop	Total Accessions	NSSL Only
Guar	413	888
SP Legumes	2,906	91
Trifolium	1,965	33
Winged Bean	166	8

Annual Clover & Special Purpose Legume Characterization Data

Crop	Total Accessions	Observation Data Loaded
Guar	413	1,890
SP Legumes	2,906	5,600
Trifolium	1,965	9,393
Winged Bean	166	50

Curatorial Accomplishments

- 50 pollination cages provided efficient regeneration for cross-pollinated clover accessions including berseem, persian and crimson clovers. Characterizations were recorded.

- 70 self-pollinated legumes with novel value-added phytochemical traits were regenerated under field conditions. Characterizations were recorded.

Curation/Research Objectives

- Collaborative curation strategy development.
- Molecular marker discovery.
- Collaborative evaluation of legumes for nematode reduction.

Curation/Research Accomplishment

- A curation strategy developed by myself and Dr. Stephanie Greene, Washington State University using clover as an example. The strategy utilizes the crop gene pools, classification, and geographical distribution of the species, new crops and new uses, and genetic erosion.

Molecular Marker Discovery

- Genomic DNA has been extracted from about 150 subclover accessions.
- AFLP markers in conjunction with morphological notes will be used to ascertain potential redundancies within the collection.

Use of Legume species as a Soil Amendment to Reduce Root-Knot Nematode Populations

- Both *Indigofera spicata* and *I. suffruticosa* when added as a soil amendment at the high rate reduced nematode galls by ≥ 90%.

Curation Work in Progress

- 25 cross-pollinated clovers (including crimson and arrowleaf) plus 25 self-pollinated clovers (including relatives of crimson, arrowleaf and subclover accessions) were planted in the field last Fall with concurrent quality plant growth for regeneration. Characterizations are being recorded.

- 100 legumes will be regenerated in the field also. These will be characterized at the appropriate time when plants have reached about 50% maturity.

Curation/Research in Progress

- AFLP and morphological data will be utilized to identify potentially redundant accessions in the subclover collection.

Future Curation Goals

- NSSL back-ups for newly acquired accessions and needed regenerated material.

Future Curation/Research Goals

- Development of collaborative screenings of selected phytopharmaceutical species for therapeutic phytochemical variability.

- Discovery of molecular markers in *Trifolium* and other important legumes.

Appendix 5

Grasses, Sorghum, and Miscellaneous Crops

Personnel

Gil Lovell, Agronomist & Curator
Donnie Hice Curatorial Support

Warm Season Grasses Collection

- Total Accessions 6,995
- Number backed up at NSSL 1,357
- Number not available 5,947

Grasses Curatorial Activities

- In a 5-year projection in 1997, plans were to regenerate 500 PI's per year
- In 1998 the goal was reduced to 300 PI's because of reduced available man-hours
- In 1999, 162 PI's will be regenerated
- At the rate of 300 accessions per year it will take 3 years to regenerate the 1,008 that are currently "not available"
- After 3 years at this rate, we would still have 4,590 accessions requiring regeneration to provide seed for backup at NSSL

Sorghum Collection

• Total accessions	29,914
• Number backed up at NSSL	23,749
• Number not available	1,920
• Core collection	2,443

Sorghum Major Concerns

- The position of Sorghum Curator which is now vacant

- The question regarding the continuing availability of the ARS unit at St. Croix, V.I. for winter nursery regenerations

Miscellaneous Crops

Bamboo, Castor, Hibiscus,
Pearl Millet, Sesame, and Miscellaneous

Pearl Millet

- Not available 48

- Forty-eight are scheduled for regeneration in the fall of 1999, if St. Croix remains available

- That regeneration should result in all accessions being available and all backed up at NSSL

Hibiscus

- To complete regeneration of the remaining 96 accessions, need $5,000 per year for the next two years

- Currently the cost of $5,000 per year is covered by the $10,000 per year allocated to the Grass Curator for “operations”

Needs

- Most critical need, additional man-hours in upgrading status of Grass Collection
  - Accelerate the rate of regeneration significantly (i.e. 600 - 1,000 accessions per year)
  - Requires an additional 3 FTE's per year assigned to Field Services

- Cost of first year for additional manpower to upgrade status of Grass Collection
  - Minimum of $63,000

- Followed by a projected cost increase of 3 - 5 % per year

- Access to winter nursery operations in a tropical area

- St. Croix needed for:
  - Sorghum
  - Pearl Millet
  - Cowpeas

- With corn also being grown at St. Croix, there isn't enough capacity for all our other needs

- Need funding to use contract services with the University of Puerto Rico or some other suitable tropical location

- Funding to provide for Seed Lab testing of grass accessions to determine seed viability (% germination)

- Knowing germination provides for better ranking of regeneration schedules

Needs continued

- Savings can be realized by avoiding regeneration
- Average cost per accession to regenerate is $140.00
- Cost of germ test is approximately $10 - 12 per accession

Appendix 6

Pathology and Vigna Curation

Personnel

Graves Gillaspie, Research Plant Pathologist & Curator
James Chalkley, Curatorial Support

Dave Pinnow, Pathology Services

Objectives

- Develop and apply new technologies for detection, characterization, and elimination of pathogens on introduced plant germplasm.

- Curate the National Plant Germplasm System *Vigna* germplasm collection including acquisition, maintenance, evaluation, and distribution.

- Test introduced germplasm for the presence of pathogens and conduct therapeutic procedures to eliminate such pathogens prior to the release of the germplasm.

Vigna Collection Status

Availability

CROP	TOTAL ACCESSIONS	NUMBER AVAILABLE
Cowpea (*V. unguiuclata*)	7,853	4,921
Mung bean (*V. radiata*)	3,889	3,802
Urd bean (*V. mungo*)	300	288
Miscellaneous spp.	594	296

Backed Up

CROP	TOTAL ACCESSIONS	BACKED UP AT NSSL
Cowpea (*V. unguiuclata*)	7,853	5,492
Mung bean (*V. radiata*)	3,889	3,802
Urd bean (*V. mungo*)	300	288
Miscellaneous spp.	594	296

Observation Data

CROP	TOTAL ACCESSIONS	OBSERVED DATA LOADED
Cowpea (*V. unguiuclata*)	7,853	34,887
Mung bean (*V. radiata*)	3,889	16,945
Urd bean (*V. mungo*)	300	1,690
Miscellaneous spp.	594	1,348

Core Collections

CROP	TOTAL ACCESSIONS	CORE
Cowpea (*V. unguiuclata*)	7,853	699
Mung bean (*V. radiata*)	3,889	410
Urd bean (*V. mungo*)	300	
Miscellaneous spp.	594	

Accomplishments

- Greenhouse grow out of 50 cowpea lines previously put through greenhouse virus elimination, but failing to produce seed in Georgia field regeneration tests.

- Regeneration of 157 lines of cowpeas in the field/greenhouse at Griffin.

- A manuscript has been accepted on a PCR-based detection method for cowpea mottle virus in cowpeas.

- Work completed and published on a strain of peanut stunt virus found in *Desmodium* sp. in regeneration plots in Griffin.

- A manuscript has been accepted for publication comparing regeneration of photoperiod-sensitive cowpeas at sites in Georgia, Puerto Rico, and St. Croix. The Puerto Rican site produced the best yields with very little virus contamination.

- Work has progressed on the development of IC-RT-PCR methods for detection of peanut stripe, peanut mottle, and a Brazilian potyvirus in peanuts.

Needs

- Technical support for molecular genetic analysis of core collections and funding for supplies.

- Location at which to do regeneration of photoperiod-sensitive cowpeas.

- Increased funding for regeneration of collection so that more support personnel can be hired.

- Funding for seed viability studies.

SERVICE PATHOLOGY ACTIVITIES

PEANUTS

- 158 P.I. lines tested as seed for PStV before regeneration planting

- 1,050 P.I. lines growing in the Byron increase field inspected three times for symptoms of PStV infection

- 489 plant samples run for one or more of 5 different viruses in greenhouse increase, quarantine screening, screening of wilds (as transplants) before field increase planting, field inspection testing, or research testing

VIGNA

- 532 samples tested for 4 viruses in greenhouse increases

- 133 samples from field increase plots tested for 7 viruses

- 466 samples from greenhouse-grown plants from seed increased in St. Croix tested for 4 viruses

- 480 samples from greenhouse-grown plants from seed increased in Mayaguez, P.R., tested for 4 viruses

- 644 samples virus-tested for various research projects

OTHER LEGUMES

- 33 clones from tissue culture screened for SPFMV and SPVD

Appendix 7

PLANT GENETIC ANALYSIS

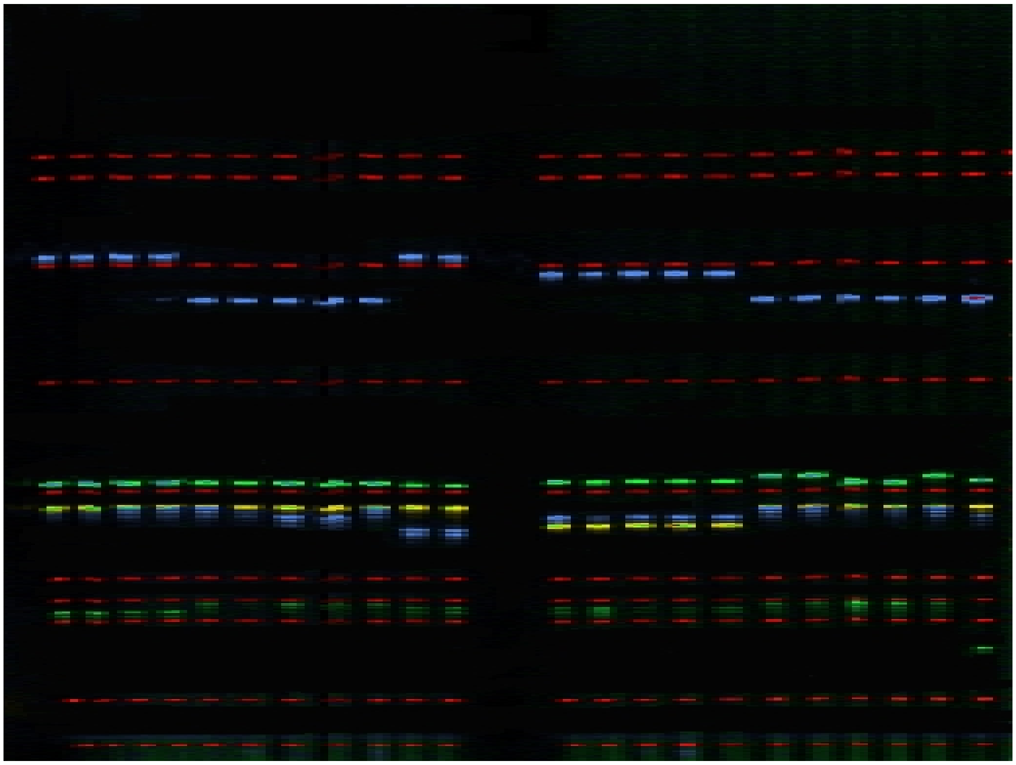
Personnel

Dr. Rob Dean, Research Geneticist

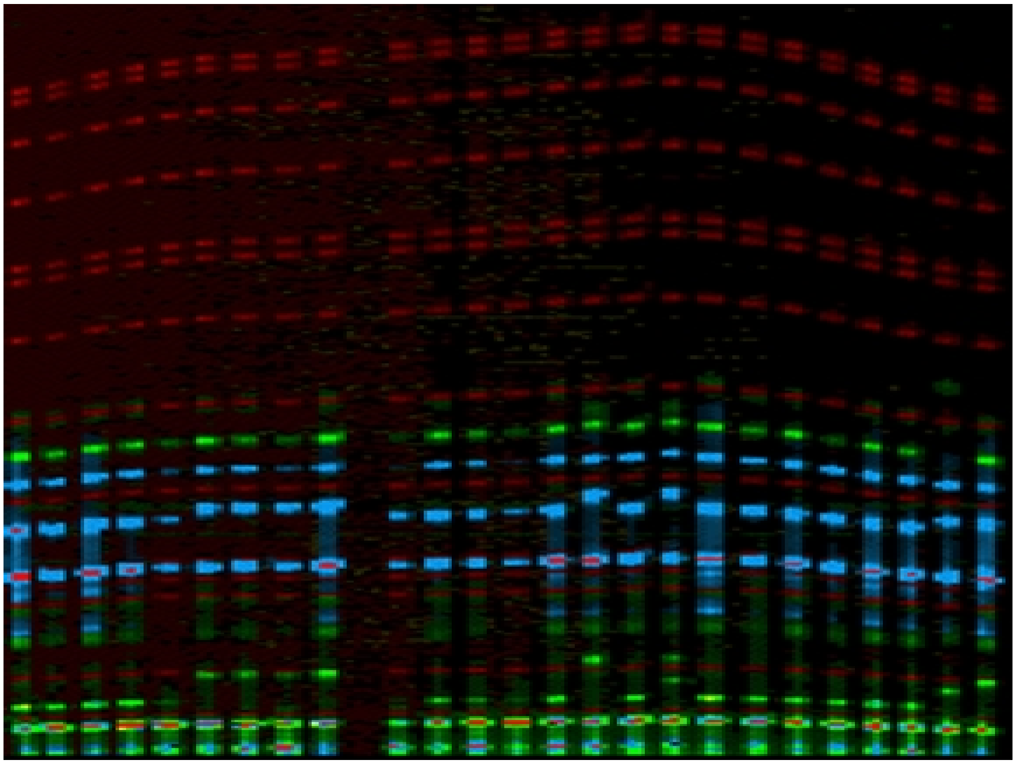
Mark Hopkins, Plant Pathologist
Sue Kilgore, Lab Assistant

SSR Markers

Sorghum SSR markers – 15 markers divided into 3 multiplex sets. These markers were then used to access levels of among, within, and between variation in Sorghum ‘Orange’



Peanut SSR markers – discovery of characterization of 7 polymorphic markers



Accomplishments

- Paspalum SSR markers were obtained from an enriched library and tested for polymorphisms.
- Seven were used in two multiplex sets to examine between and among levels of variation.

Current Objectives

- Use the SSR markers in peanut to determine their ability to separate accessions into botanical type.
- Determine amount of genetic variation between and within peanut accessions.
- Sequence peanut cDNA clones for additional polymorphic SSR markers.
- Determine amount of genetic variation among, between and within clover accessions.
- Develop the ability to normalize peak height within and between gels

Future Objectives

- Assess peanut core collection for genetic diversity
- Utilize SSR markers discovered in Paspalum sp. to assess turfgrass collection
- Determine whether published SSR markers for Vigna are reliable to assess collection
- Clone cDNA libraries, sequence, and establish a database from major crop species in our collection
- Continue to explore new molecular technologies and marker systems for their utilization by plant curators.

Future Needs

- Enhanced storage for accessing database from a web interface to the SunSparc.
- Hardware and software upgrades for the ABI 373 and 377.

Appendix 8

NSSL Report

United States Department of Agriculture

Research, Education, and Economics
Agricultural Research Service

Mission
Plant Germplasm Preservation Research Unit
National Seed Storage Laboratory

*Increase the longevity of samples stored in the base collection

Optimum water contents
Liquid nitrogen
Pre and post harvest treatments

*Increase the number of species that can be stored in the base collection

Recalcitrant seeds
Cultures of non-seed producing plants
Pollen

*Increase our ability to preserve elite genetic combinations

Preserve clonal materials
Identify populations/individuals giving maximum heterogeneity

*Increase the efficiency of monitoring viability

Automated germination assays
Non-invasive viability tests
Predictive models
Seed quality factors

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Proposal for New Category I Scientist Position
(vacant following reassignment of Dr. Eric Roos)

There are several disciplines lacking in the PGPRU that would help round out our program. Our biggest need was one of accountability: how do we know that we are representing and maintaining the genetic diversity of agronomically important crops?

We feel there is a gap between the information gleaned from the Plant Genome Project and its utilization within the National Plant Germplasm System. Gene mapping is critical to the understanding of the genetic architecture of species and the identification of important genes for crop productivity, tolerance to biotic and abiotic stresses, and value-added characteristics. However, an assessment of the genetic diversity for these traits, within species and within the NPGS collections, is not centralized. We propose to fill that gap in the PGPRU with a **Plant Population Geneticist** who uses molecular genetic approaches.

The scientist in this position will be responsible for developing a research program to identify and measure the genetic diversity of specified crops and the representation of that diversity in the NPGS collections. The research will involve assessments of overall genetic diversity, as measured by neutral markers, and evaluation of the allelic diversity of important genes and gene combinations identified through the Plant Genome Project. While the Plant Genome Project handles only a few model species, our strength is that we have access to many species -and many populations within a species. The overall objective is to ensure that the NPGS collections have adequate representation of the genetic diversity of species and that this diversity is characterized and maintained.

The proposed position fits into the present mission of the PGPRU: to increase our ability to preserve elite germplasm by identifying populations or accessions that give maximum heterogeneity, particularly for valuable genes. It will also augment research on the conservation of germplasm of other organisms.

PLANT PHYSIOLOGIST, GS-435-11

A. Introduction

Incumbent participates as a responsible team member of the Plant Germplasm Preservation Research Unit located at the National Seed Storage Laboratory in Fort Collins, Colorado. Research conducted by the PGPRU emphasizes long-term preservation of plant germplasm. The incumbent performs a wide variety of tasks associated with preservation and maintenance of germplasm for long term storage.

B. Major Duties

The incumbent serves as a specialist in seed physiology and participates with the supervisor and colleagues in developing plans and studies long-term conservation of plant embryos with unknown, recalcitrant or intermediate physiologies. The incumbent will help to establish a pilot project to incorporate recalcitrant and intermediate seeds growing in NPGS clonal repositories as well as those growing in wild populations in threatened habitats within the US into the base collection at the NSSL using established cryopreservation protocols.

Identifies the level of desiccation and freezing tolerance of mature embryos from a wide number of species for which this information is unavailable.

Develops germination and culture assays that relieve embryo dormancy

Adapts and modifies existing cryopreservation protocols for use with embryos with different physiologies

Establishes a database for the new collection, documenting species, geographical origin, population and lineage information, growth habits, viability assays and results, and storage protocols, and location in storage. Makes database compatible with those used by GRIN, CPC database and KEW Gardens.

Transfers technology to clonal repositories and smaller genebanks that work with recalcitrant and orthodox seeds.

Analyzes, interprets and summarizes experimental data relying on computer and statistical based skills. Incumbent is expected to have knowledge of appropriate experimental design and proper means of analyzing data.

Reviews background material through literature searches in scientific and trade journals. The incumbent tests any new methods or techniques which may help in the analyses.

Contributes to the preparation of manuscripts, reports, abstracts and other publications.

C. Factors

1. Knowledge Required

Knowledge of seed anatomy and physiology

Knowledge of tissue culture techniques with particular emphasis on embryo culture

Knowledge of anhydrous biology and cryobiology

Knowledge of principles of insitu and exsitu germplasm conservation

Knowledge of safe laboratory procedures and practices

Knowledge of database organization

2) Supervisory Controls

Incumbent works under the general supervision of a GS-14, Plant Physiologist who defines the overall direction of the research. The incumbent participates with supervisor in deciding priorities of specific projects. Specific projects are frequently very broad in scope. The incumbent has primary responsibility for planning, conducting and completing the various phases of work. The incumbent selects approaches, methods and modifications in techniques to facilitate work. The incumbent is responsible for keeping the supervisor informed of work load, major problems and any methodology impacting the research.

3. Guidelines

Guidelines include instrument manuals and method from scientific and technical journals, handbooks, published techniques, established protocols and previous training and experience. These guidelines and not always applicable. The incumbent, therefore, makes creative adaptations and frequently modifies established methodology to each new experiment. The incumbent must exercise judgement in selecting the best approach or methodology, and in making those modifications necessary to solve specific problems or to meet specialized requirements.

4. Complexity

The assignment requires that the incumbent draw upon knowledge of several disciplines and skills including plant physiology, seed technology and biochemistry and biophysics. The incumbent must apply complex concepts of experimental procedures to overcome technical difficulties and to obtain reproducible results. In addition, the work requires modification of existing approaches or development of new approaches to achieve project goals.

5. Scope and Effect

The expertise provided by the employee affects the overall program of the National Seed Storage Laboratory, which serves a vital role in preserving plant germplasm for future generations. The ability to provide clear, reliable and valid results and conclusions will enhance preservation of genetic material.

6. Personal Contacts

Contacts are with other staff members at the National Seed Storage Laboratory, with visitors to the research unit and with researchers in similar areas at Colorado State University, NPGS and plant conservation organizations.

7. Purpose of Contacts

Contacts with research scientists are to set priorities in preserving plant species and to exchange information and devise strategies for culturing techniques and cryopreservation protocols. Other contacts are necessary for maintaining laboratory supplies and equipment.

8. Physical Demands

The work involves moderate physical activity that requires average physical condition, hand-eye coordination, manual dexterity, and mental alertness.

9. Work Environment

The majority of the work is performed in a laboratory setting. Organic solvents, acids, caustic and toxic materials are sometimes used.

Liquid nitrogen is used regularly. Laboratory safety procedures are mandatory.

United States Department of Agriculture

Research, Education, and Economics
Agricultural Research Service

June 4, 1999

SUBJECT: NSSL 1998 Progress Report

TO: Regional Technical Advisory
Committees on Plant Germplasm

FROM: National Seed Storage Laboratory
Steve A. Eberhart, Director
Christina Walters, Research Leader
Loren E. Wiesner, Research Leader

ADMINISTRATION
Steve A. Eberhart, Director

A national program to preserve genetic resources of animals, insects, and microbes, in addition to plants, was authorized by the 1990 Farm Bill. Expanding the NSSL mission to include research and long-term backup storage of these other valuable genetic resources has great merit. An ARS Task Force has been appointed to evaluate this proposal.

Dr. Eric Roos was selected as the Assistant Area Director for the Northern Plains Area effective March 14, 1999. Dr. Christina Walters is now the Research leader for the Plant Germplasm Preservation Research Unit.

Regeneration of endangered Latin American maize landrace accessions will continue in a cooperative project with 13 countries under a Specific Cooperative Agreement with CIMMYT. NSSL received 1,744 samples in 1998 bringing the total to 13,380 maize landrace accessions regenerated and stored under this project.

The MOU with IRRI for long-term storage of rice security backup samples has been renewed.

SEED VIABILITY AND STORAGE RESEARCH UNIT
Loren E. Wiesner, Research Leader

A total of 26,514 samples were placed into storage during 1998. Of the samples stored, 20% were placed in cryostorage and 80% in -18°C conventional storage. NSSL received 20,360 new samples in 1998: 403 seed samples from Plant Variety Protection office, 412 clonal materials including 354 apple buds, 25 endangered species seed samples from Botanical Gardens, 39 plant quarantine seed samples, 15,044 seed samples from Regional Plant Introduction Stations, 220 seed samples for Crop Science Registrations, 1,744 corn samples from the Latin American Maize Regeneration program, 253 tomato samples from Charles Rick collection, 283 maize genetic stocks, 1,464 seed Security Backup samples from the National Center for Agricultural Utilization Research, and 473 seed samples from other individuals and organizations.

A total of 2,781 accessions were distributed to 15 countries and 114 scientists. Included in these distributions were 816 sorghum, corn, and pearl millet quarantine samples sent to St Croix for regeneration.

Studies were conducted to develop germination procedures for apple and grape seed. Studies were continued on evaluation of long-term storage of *Rhizoctonia* in liquid nitrogen, on methods of breaking dormancy of sunflowers for field emergence, and on identification of duplicate accessions in the pea collection.

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The following countries increased and shipped maize seed to CIMMYT: Bolivia 35 accessions, Brazil 208 accessions, Ecuador 168 accessions, Mexico 444 accessions, Peru 82 accessions, and Venezuela 8 accessions. CIMMYT assigned serial numbers and shipped 1,410 accessions to NSSL for security backup storage. A meeting of Principal Investigators participating in the maize regeneration project was held June 1-5, 1998 at CIMMYT headquarters to coordinate efforts among countries.

A LAMP core subset has been developed by CIMMYT staff. The list and the data set of the LAMP core subset will be published in a CIMMYT special publication and will be available on a CD-ROM. The Caribbean race core subset was published in Crop Science 38:1378-1386.

During the past year 1,252 accessions were removed from storage and tested for germination. Sugar beet and tomato were the two species evaluated. Our retest schedule is being determined based on the storability of each crop. We have determined that onion, lettuce, carrot, pepper, peanut, clover, sorghum, rye, bluestem, tomato, tobacco and beet should be the first crops to be retested as they do not store as well as other species.

Special seed increases were supported for accessions of rye landraces and wild relatives in Poland and for accessions of wild potato species in Peru. Twenty-one Capsicum accessions were collected in Paraguay and placed in security backup at NSSL.

SVSRU personnel conducted 56 tours of the Laboratory for 634 individuals. These tours were for grade school, high school and college students, girl and boy scouts, and 4-H members, in addition to scientists and the general public.

PLANT GERMPLASM PRESERVATION RESEARCH UNIT
Christina Walters, Research Leader

The following report summarizes research activities by the scientists in the Plant Germplasm Preservation Research Unit (PGPRU) at NSSL. Each project is briefly summarized and includes personnel assigned, the problem area, approach, and results for the past year. Following the reports is a list of publications appearing in print in 1998-99.

PERSONNEL: In March 1999, Dr. Eric Roos resigned as Research Leader of the PGPRU to become Assistant Area Director (NPA). He had been serving as Acting Assistant Area Director since June 1997. Dr. Christina Walters replaced Dr. Philip Stanwood as Acting Research Leader in October 1998, and she has recently been selected as the Research Leader. In November 1998, Ms. Jennifer Crane joined the research staff in a new Research Support Scientist position. Ms. Crane is initiating a pilot program to cryopreserve embryos of temperate trees and tropical fruit crops. Dr. Darren Touchell continues as a Postdoctoral Research Associate studying genetic conservation of wild and cultivated populations of the endangered species *Zizania texana*. A new Plant Physiologist/Molecular Biologist position was announced in February 1999. Research in this position will focus on enhancing our ability to cryopreserve vegetative tissues by determining environmental and developmental switches that enhance plant cell tolerance of desiccation and freezing stresses. Selection of the candidate will be made by July 1999.

VISITING SCIENTISTS: Ms. Paula Power (US Fish & Wildlife-Texas) visited for two weeks in July 1998 to study embryogenesis and desiccation tolerance of Texas wild rice, *Zizania texana*. Mr. James Wesley-Smith (University of Natal, Durban So. Africa) is on a year's sabbatical (beginning August 1998) to develop technology for ultra-rapid cooling rates. Mr. David Merrick (Kings Park Botanical Garden, Perth Australia) studied the physiology and water status of seeds from native Australian species at the NSSL in October 1998. Mr. Alvin Yoshinaga (Lyon Arboretum, Honolulu) visited for one week in February 1999 to discuss collaborative work on the physiology and storage behavior of seeds from endangered Hawaiian flora. Ms. Mirian Eira (EMBRAPA/CENARGEN Brasil) finished her doctoral research on the storage behavior of Coffea spp. seeds and will be defending her thesis in July 1999 in Brasilia. We received the usual large number of visitors from all over the world for periods of 1 to 2 days.

GRADUATE STUDENTS: Mr. Jian Fang, from the Peoples Republic of China continues his doctoral thesis research on damage to seeds during ultra-dry storage (advisor: Dr. Roos). Ms. Kim Davidson continues her doctoral thesis research on the loss of desiccation tolerance during seed germination (advisor: Dr. Walters). Ms. Terri Christensen is writing her dissertation for a M.Sc. degree on the germination results of seeds from native California species stored for 50 years (advisor: Dr. Roos). Mr. Robert Cook is writing his dissertation for a M.Sc. degree on the effect of lipid composition on aging rates of soybean seeds (with Dr. Walters).

TECHNOLOGY TRANSFER: The research staff of the PGPRU routinely provides advice on the storage behavior of seeds, procedures to maximize seed longevity, cost-effective methods to process seeds for storage, and methods to germinate seeds. The PGPRU was represented at the annual meetings of the Regional Technical Advisory Committees, Plant Germplasm Operations Committee, American Society of Plant Physiologists and American Society for Horticultural Science in 1998. Also this past year, research was presented at a Gordon Conference (Temperature Stress in Plants (2/99)) and international meetings in New Zealand (3/98), France (6/98), Japan (10/98), Malaysia (10/98) Mexico(1/99), and Denmark (3/99). Dr. Walters continues to consult with several groups (Leech Lake Reservation, US Fish & Wildlife, botanical gardens and commercial growers) to conserve species endemic to the US in ex situ collections. She continues to serve on the Science Advisory Board of the Center for Plant Conservation. In cooperation with the International Plant Genetic Resources (Rome), Dr. Walters worked to establish recommendations for seed genebanks in developing countries. Dr. Towill spent a month at the New Zealand Institute for Crops and Food to exchange information on cryopreservation techniques of plants grown in microculture. Dr. Walters spent a month at the Universite de Pierre et Marie Curie, Paris exchanging information on calorimetric techniques. Dr. Stanwood has now added about 4500 images to the GRIN database. PGPRU personnel received numerous inquiries from small companies preparing seed stuffs as a precaution against a possible Y2K disaster.

A complete list of publications, excluding abstracts, from the National Seed Storage Laboratory dating from 1960 is available for distribution. Copies of papers can be requested from: Ms. Peggy Matti, Plant Germplasm Preservation Research, National Seed Storage Laboratory, 1111 South Mason Street, Fort Collins, CO 80521-4500. We have set up a World Wide Web page on the INTERNET that can be accessed via the following: http://www.ars-grin.gov/nssl/nsslmain.html
The list of publications is also available through this web site.

FOREIGN TRAVEL: In January 1998, Dr Walters met with colleagues at the University of Reading and the International Plant Genetic Resources Institute (IPGRI) in Reading, UK to develop recommendations for seed storage in gene banks in developing countries. Dr. Towill was invited to spend six weeks at the New Zealand Institute for Crop and Food Research, Christchurch, New Zealand in February/March 1998. Dr. Walters spent six weeks in France in May/June 1998: 5 weeks at the Universite de Pierre et Marie Curie, Paris as an invited professor to collaborate on calorimetric properties of seeds and one week visiting the germplasm repositories in Montpellier, France. In October 1998, Dr. Walters was invited to present a keynote address at a meeting sponsored by the International Union of Forestry Research Organizations in Malaysia. Also that month, Drs. Towill (declined) and Touchell were invited to present papers at a meeting sponsored by Japan International Research Center for Agricultural Sciences and International Plant Genetic Resources Institute in Japan. In January 1999, Dr. Touchell, Mr. Wesley-Smith, and Ms. Eira presented research conducted at the NSSL at the VI International Workshop on Seeds in Mexico. In March 1999, Dr. Walters was invited to participate in a Danish-sponsored symposium on seeds in Copenhagen. Dr. Walters plans to travel to Brazil to attend the thesis defense of Ms. Eira and present at the annual meetings of the Brazilian Plant Physiology Society.

THE OPTIMUM WATER CONTENT FOR SURVIVAL OF DRIED GERMPLASM

CHRISTINA WALTERS (Plant Physiol), Jennifer Crane (Research Support Sci), Lisa Hill (Biol Sci Tech), N. Kameswara Rao (ICRISAT, India), Hu Xiaorong (ICGR, CAAS, China), Jan Engels (IPGRI, Rome), Julia Buitink (Agricultural U, the Netherlands), Mirian Eira (EMBRAPA, Brazil)

PROBLEM: Germplasm must be stored at precise water contents to maximize longevity. These water contents vary among species and with storage temperature. Clearly, there are insufficient resources to determine the optimum water content for all species represented in the NPGS. We are using thermodynamic principles as a tool to predict optimum water contents. This research is funded in part by the International Plant Genetic Resources Institute (IPGRI), Rome.

APPROACH: We have studied phylogenetically diverse organisms, pollen, and more than 30 species of seeds to determine the interaction of water content, temperature, relative humidity, and deterioration rates.

Water properties in cells as a function of water content and temperature were also measured using sorption isotherms or differential scanning calorimetry. These thermodynamic properties are correlated with the optimum water content. Aging experiments are long-term, some with more than nine years of storage data.

RESULTS: We have identified two classes of desiccation tolerant organisms, and the optimum water content for storage varies among the classes. Many organisms survive the short-term effects of complete dehydration (examples are many crop seeds, *Artemia* cysts, some microflora). The optimum water contents for storage of these extremely desiccation tolerant organisms correspond to about 15-22% RH, regardless of the temperature, species, or tissue. A second class of organisms (seeds with this physiology are often called "intermediate") survive drying to as low as 20% RH, but the optimum moisture level for storage is about 55%. Above and below the optimum humidity, organisms deteriorate faster. This means that there is a limit to the beneficial effect of drying seeds, and once the water content is optimized, reducing the temperature is the only way of prolonging seed storage life. Drying protocols can be easily established to obtain optimum storage conditions for any storage temperature used. This research has enabled us to predict the best moisture conditions for seed storage and to expedite the preparation of seeds for storage. Storing seeds under optimum conditions will ultimately limit the frequency at which samples are monitored and regenerated. These conclusions were published in a letter to NATURE (Sept, 1998). In addition, Dr. Walters was guest editor of a special issue of SEED SCIENCE RESEARCH (September 1998) which summarizes the current knowledge of seed storage practices.

THE KINETICS AND MECHANISM OF DETERIORATION IN DRIED ORGANISMS

CHRISTINA WALTERS (PI Physiol), Lisa Hill (Biol Sci Tech), Ming Zhang (post-doctoral research associate, China), Julia Buitink (Agricultural U, the Netherlands), Mirian Eira (EMBRAPA, Brazil), Robert Cook (M.Sc. student, CSU)

PROBLEM: Organisms that survive drying can be placed in "suspended animation" and remain viable for a long time. However, all organisms eventually die. The inevitable loss of viability presents a problem for genebanks since it necessitates monitoring germplasm and periodically regenerating it. Genebank operators need to predict which samples are more susceptible to deterioration and to know how to prevent deterioration. To address these needs, we must elucidate the mechanism(s) of deterioration during storage and the precise relationships among the kinetics of aging, the temperature and relative humidity of storage, and intrinsic properties of cells.

APPROACH: Thermodynamic properties of water in seeds are measured since they appear to correlate with the nature and kinetics of deterioration reactions. We are currently trying to evaluate the use of volatile emission from seeds as a non-destructive assay of seed aging rates. Once reliable estimates of aging rates under a variety of storage conditions are known, we can produce predictive models for storage longevity and begin studies of the environmental and genetic components of seed quality that affect seed aging rates. Ultimately, this will enable us to find the underlying chemical or physical properties of seeds that give rise to the quality factors.

Results: We have developed phase diagrams for seeds based on calorimetric measurements of water. We have established that the optimum moisture content for seed storage corresponds to a change in the heat capacity of water, but not to so-called glass transitions. At water contents below the optimum, lipid peroxidation reactions appear to dominate and the kinetics of these are presently being described. At water contents above the optimum, reactions involve glycolysis and unregulated respiration. The effect of temperature on these reactions can be described predominantly by Arrhenius behavior with apparent activation energy similar among tissue types. This information gives us powerful tools to predict aging rates for different seed species and lots.

DEVELOPMENTAL PROCESSES DURING EMBRYOGENESIS AND GERMINATION THAT LEAD TO CHANGES IN DESICCATION TOLERANCE, STORAGE LONGEVITY, AND CRYOSTABILITY

CHRISTINA WALTERS (PI Physiol), J. Crane (Biol Sci Tech), L. Hill (Biol Sci Tech), K. Davidson (Ph.D. student, CSU), P. Berjak (U of Natal, So. Africa), N.W. Parnmenter (U of Natal, So. Africa) J. Farrant (U of Cape Town, So. Africa), F. Corbineau (U P.M. Curie, Paris), D. Come (U P.M. Curie, Paris), C. Bailley (U P.M. Curie, Paris), J. Wesley-Smith (U of Natal, So. Africa)

PROBLEM: Embryos acquire, to varying degrees, the ability to survive the immediate (desiccation damage) and long-term (aging damage) effects of desiccation during development. They lose this ability when they germinate. Many economical ly-important species from tropical areas produce seeds that have limited abilities to survive drying and storage. Our task is to understand the basis of the limitations, first at a physiological level and then at a genetic level. We believe that this information will allow us to successfully cryopreserve all embryos-somatic or zygotic-and perhaps to artificially enhance the desiccation tolerance of other plant propagules that we wish to cryopreserve.

APPROACH: We have started to "map" out the developmental changes in seeds of diverse phylogenetic backgrounds on a biophysical, chemical, and ultrastructural basis and to determine which changes lead to greater tolerance of desiccation.

RESULTS: We have shown that as embryos mature there is almost a continuous decrease in the critical water content at which desiccation damage occurs. This has led to the idea that desiccation tolerance is a purely quantitative feature. However, we have shown that there are only a few critical water potentials (-1, -3.5, -12, -50 MPa), and developing embryos approach these in discrete steps. We believe this is an important finding as it allows us to search for developmental switches and the genetic basis of desiccation tolerance.

DEVELOPING CRYOPRESERVATION PROTOCOLS FOR HYDRATED EMBRYO TISSUES: THEORY OF COOLING RATE

CHRISTINA WALTERS (PI Physiol), J. Wesley-Smith (U of Natal, So. Africa)

PROBLEM: Developing embryos and fully mature embryos of some species are not tolerant of desiccation and must be preserved in the hydrated state. Cryopreservation in liquid nitrogen is the only alternative. To prevent ice crystals during cooling to liquid nitrogen temperatures, cryo-biologists either add cryoprotectants (or exploit the natural cryoprotectants present in cells) or cool extremely rapidly to prevent the formation and growth of ice crystals. Because most of the propagules we work with are large (>500,000 cells), they cannot be cooled using methods developed for other organisms.

APPROACH: Our goal is to develop technology to cool larger propagules (500,000 cells) sufficiently rapidly to prevent lethal ice damage. There are several factors that determine the necessary cooling rate: the thermal mass of the propagule, the intrinsic level of cryoprotectants in the cell, the level of cell differentiation, and how large ice crystals can be without causing damage. We will consider all of these factors in a multidisciplinary study using electron microscopy, differential scanning calorimetry, seed physiology, and tissue culture. Cooling rates are controlled by a spring loaded-plunging device that shoots propagules at different speeds into various depths of different cryogens.

RESULTS: Cooling rates of up to 100OC/sec have been achieved. Embryonic axes that are slightly dried have a larger window of allowable cooling rates, and close to 100% survival has been achieved for numerous species. Embryos that are fully hydrated have a small window of allowable cooling rates. We have shown that ice crystals can form in cells with no apparent damage and are currently evaluating the size of those crystals.

EVALUATION OF PLANT GERMPLASM USING DIGITAL IMAGE ANALYSIS AND CRYOPRESERVATION OF PLANT GENETIC RESOURCES

PHILLIP C. STANWOOD, (Res Agron); Lana Wheeler, (Biol Sci Tech)

PROBLEM: Seeds are used as the primary means of preserving plant diversity for future generations. From a practical point of view, seed moisture content and storage temperature are the two primary factors that one can modify to lengthen the time that seeds can be preserved. The use of ultra-cold temperatures for storage, cryopreservation (LN_2 -196°C), has been suggested as a means of greatly extending the storage life of seeds and other biological materials. Short-term studies (< two years) have demonstrated the efficacy of seed cryopreservation on more than 130 species. However, longer-term responses are needed to evaluate the full potential of this technology. A significant problem is how one evaluates and monitors the deterioration of seeds over time. Seed germination has been, and is currently, used as the evaluation technique. There are certain limitations to this technique. Early detection of deterioration before loss of germination would be highly desirable, reducing the likelihood of loss of genetic diversity from reduced seed viability.

APPROACH: Current research is directed at: 1) developing technologies and understanding principles of cryopreservation of seed and pollen using liquid nitrogen (LN_2 , -196°C) as a storage medium; 2) developing and using digital imaging to measure vigor (deterioration) of seed germplasm; and 3) evaluating the concept of image oriented databases as a means of data archiving and distribution.

A robotic system based on digital image analysis is being developed to simultaneously conduct 100 seedling root-growth vigor tests. The Slant Growth Robotic (SGR2) system is temperature controlled and continuously slewing, which provides a similar micro environment to each sample being tested. The slewing activity is critical for a significant reduction of experimental variation. This reduced error allows for more reliable results, use of a smaller number of seeds per test, reduced labor and material cost, and enhanced evaluation of the seed germplasm. The output of the SGR2 system is a series of time course root-growth curves for individual germinating seeds. From these curves, analyses can be done to determine the relative vigor (deterioration) of a sample. Reduction of vigor precedes loss of seed germination; thus, identification of vigor loss provides an extremely sensitive and valuable tool in accessing the storability of a sample and thus expected longevity. This information greatly enhances the management of genetic resources, ultimately improving the preservation of the material while reducing costs and labor inputs.

A digital image oriented database concept is being investigated to enhance the collection, storage, and dissemination of information concerning our preserved plant genetic resources. Chickpea, lettuce, and sugar beet image sets are being used as test species for this part of the project. Information from these studies is consolidated and provided on photo CD-ROM. Information and images from these image databases are also placed on the USDA-ARS, Germplasm Resources Information Network (GRIN) in Beltsville, Maryland and are available through the Internet and World Wide Web (www.ars-grin.gov).

Cryopreservation of plant genetic resources using liquid nitrogen (LN_2, -196°C, -320°F), offers the opportunity to enhance the longevity and quality of stored materials such as seeds, embryos, cell suspension, pollen, and vegetative buds. This technique can also improve the reliability of the storage system and reduce costs and other resources needed per sample. Seeds from more than 130 species have been successfully exposed and stored in liquid nitrogen. Long-term preservation studies are underway to determine the practical and biological feasibility of the cryopreservation technique for seed germplasm.

RESULTS: Much of the effort in 1998 was directed at the development of the SGR2 vigor evaluation system. Several mechanical design problems in the robotic slewing system were corrected. A new "structured" light fiber optic device was installed on the SGR2. This greatly improved the distribution of light over the test area, which is needed by the digital imaging analysis software. New software routines were developed to address root cross-over detection problems. Several new species have been successfully grown on the SGR2 including bromegrass, timothy, red clover, alfalfa, orchard grass, and sorghum seed. A large experiment (150,000 images) was conducted on lettuce and onion seed to evaluate root-growth-rate standard error vs. number of seedlings.

Seed germination testing was started for 30 species stored for 20 years at 5C, -18C and about -160C (vapor phase over liquid nitrogen). Preliminary results indicate that highest deterioration is occurring at 5C (as expected). There are a few species where loss of germination is occurring at -18C; however, most species do not show a loss of germination at this temperature. Most species stored at -160C have shown no significant loss of germination. Upon completion of the germination testing, we will start to evaluate the seed materials for loss of vigor. This will give us a more sensitive measure of seed deterioration. Ten-year storage studies on lettuce and onion seed indicated no loss of germination at -18 and -160C storage; however, there were significant reductions in seed vigor for seed stored at -18C compared to -160C. The 20-year result will further clarify seed deterioration differences at the three storage temperatures.

Seed images can be evaluated to determine variations in seed size, shape, and color. Characterization of seed color has been problematic in that 16.7 million color variations are possible in most models. The use of this number of color variations in describing seed color is impractical. A modified color model using luminescence as a basis was developed for seed. Thirty-six "basic" color (luminescence) standards were selected with appropriate standard errors. Using this model, seed color from 505 chickpea genetic selections were characterized.

CRYOPRESERVATION OF WOODY, VEGETATIVELY PROPAGATED GENETIC RESOURCES

LEIGH E. TOWILL (Pl. Physiol.), John W. Waddell (Biol. Sci. Techn), Philip Forsline and Warren Lamboy (National Clonal Germplasm Repository, Geneva, NY), L.J. Grauke (National Clonal Germplasm Repository, Brownwood, TX), Charles Simon (National Clonal Germplasm Repository, Davis, CA.

PROBLEM: Long-term preservation of species that are vegetatively propagated is needed to avoid potential loss of germplasm and is a priority area for NPGS. Cryopreservation allows for safe, long-term storage which then gives clonal repositories options for minimizing costs with field or greenhouse maintenance. Apple (Malus spp.) was the first clonal species to be routinely placed into cryogenic storage at NSSL using dormant vegetative buds and the so-called two-step cooling method. Other cold hardy, woody species were also shown to survive such a method, but often in lower percentages. Certain aspects of the method still need examination. What alternatives can be used with particularly cold-tender lines? Some of these studies are ongoing and are summations from 2-3 years of work.

APPROACH: Several parameters of the dormant vegetative bud method for cryopreservation are expected to be species specific. Grape lines from the Davis, CA repository were determined to be a priority to the National Plant Germplasm System and were examined in winter 1998-9. Cut nodal sections and isolated, whole buds were examined using modifications of a dormant, vegetative bud method. Initial studies for the eventual cryopreservation of pecan were begun. Hardiness levels of three lines of pecan were determined at three dates during the dormant season using an oxidative browning test for samples slowly cooled to subzero temperatures. Differential thermal analysis was used to determine the freezing temperature in buds which may correlate with relative extent of cold hardiness among 100 pecan lines from the Brownwood/Somerville TX locations.

RESULTS: Hardiness levels were determined by cooling nodal sections from three lines of grapes harvested from Davis, CA to -30C. Buds survived amongst the three lines to about -18 to -21. Cambial and wood parenchyma hardiness was greater than bud hardiness. Storage at -3C for several weeks increased bud hardiness about 3C. Buds removed from desiccating nodal sections (ca 12 mm in length) were about 2-5% lower in moisture content (FW basis) than that of the stem. Differential thermal analysis (DTA) using both thermocouples and thermoelectric modules showed that undried buds exhibited low temperature exotherms in the range of -15 to -25C. This confirms that grape vegetative buds supercool and do not freeze-dehydrate extensively during slow cooling. Desiccation below about 24% moisture for the whole nodal section was damaging to the bud, but the large and variable diameters of the canes and somewhat lower bud moisture content from these sections precluded a precise determination. Samples of undried or partially desiccated (ca. 20-24%) whole nodal sections from Davis materials were cooled to LN vapor from either -3, -18, -21, -24C. Survival after ca. -160C treatment was very low. Because retrieval of cryo-treated samples will probably be via culture, isolated bud complexes were used for tests. Desiccation alone (ca. 20% moisture content, FW basis) gave some survival from hardier hybrid lines (from Fort Collins, CO). Exposure to sugar followed by desiccation improved survival after low temperature exposure in lines from Davis, CA.

Three pecan lines from Texas, selected to represent tender, moderate and cold hardy lines, could be distinguished using a slow cooling procedure with nodal sections and an oxidative browning viability assay. DTAs of 100 lines from Texas showed distinct low temperature exotherms, probably due to supercooling of xylem ray parenchyma. The position of the exotherm and its pattern are being used to classify cold hardiness of the 100 lines. Correlations with field observations of hardiness will then be done. Similar studies are planned for the winter of 1999-2000. This information will be useful in determining which cryopreservation procedure might be most successful in obtaining survival after LN exposure.

CRYOPRESERVATION OF SHOOT TIPS USING VITRIFICATION METHODS

LEIGH E. TOWILL (PI Physiol)

PROBLEM: Vitrification, a method to cryopreserve diverse cells, tissues, and organs, has been shown by us and others to be effective for a range of species. This still is a relatively new method and is, as yet, not used as a routine method for cryopreservation. Vitrification is a process containing a series of steps which must be optimized. Modifications of the vitrification process usually need to be explored to develop an efficient, effective procedure. When incorporated, cryopreservation will allow clonal repositories options for cost savings in managing their field, greenhouse, or in vitro collections. We continue to investigate aspects of vitrification for several species.

APPROACH: Most studies used axillary buds excised from plants maintained in vitro. Such systems are axenic and minimize contamination when the treated axillary buds are cultured to produce the shoots. Both in vitro stock plants and the buds isolated from them may be treated prior to cryopreservation. Both liquid-based and encapsulation-based vitrification procedures are being examined. Most studies have been with sweet potato, given our previous work and observations of considerable variation in survival after cryopreservation.

RESULTS: Personnel changes during the year delayed progress in approaching some studies. Crop priorities were reevaluated for materials that would use in vitro culture, either as in vitro stock plants and in vitro retrieval or greenhouse/field plants with in vitro culture. Sweet potato *Ipomoea* spp.), potato (*Solanum* spp.), grape, garlic and pineapple will be emphasized. Culture systems are being tested to provide better stock materials for shoot tip isolation.

CRYOPRESERVATION OF SHOOT TIPS FROM *MENTHA* SPP.

LEIGH E. TOWILL (PI Physiol), Hyla Schreurs (federal, temporary appointment)

PROBLEM: The cryopreservation of clonally propagated lines provides a backup should stock plants be lost. The mint industry in the US is based on a few lines with known production potential and oil quantity/quality. The development of a backup system for the foundation stock program would be beneficial should loss occur. This backup is desired for micropropagated (test tube) and greenhouse stocks. The greenhouse stock presents challenges because of endogenous bacteria. The desire is to maintain the endobionts and not alter the stock plants since it may be argued that the presence of these endogenous bacteria contribute to the field performance characteristics. This project is partially funded by the Mint Industry Research Council.

APPROACH: Initial tests will determine whether we can use an antibiotic to suppress external growth of microbes from shoot tips or small nodal sections when samples are cultured on normal culture media. We will examine more recent methods of cryopreservation using PVS2 as a vitrification solution and glycerol preculture. Mint plants readily over-winter if acclimated, and we will examine whether acclimation can be induced in in vitro plants for achieving higher levels of survival after cryogenic treatment. We will also examine aspects of rate of cooling and warming on survival.

RESULTS: Antibiotic tests have centered on the use of PPM, but have been disappointing since levels that suppress (but not eliminate the bacteria) also retard growth. Several modifications of vitrification procedures have been tested. Preculture with 2M glycerol and 0.4M sucrose for 1-3 hours was beneficial. With this system, rapid cooling with shoot tips on foil strips gave better survival than cooling within vials. Several methods seem to give approximately similar levels of survival.

LONG-TERM PRESERVATION OF CLONALLY-PROPAGATED TURFGRASS SPECIES.

LEIGH E. TOWILL (Pl. Physiol.), H.G. Hughes (Prof. of Horticulture, Colorado State Univ.)

PROBLEM: Bermudagrass, zoysiagrass, saltgrass, and buffalograss are clonally propagated and methods are desired to avoid loss of selected species. Cryopreservation offers the potential for backup but no studies have utilized shoot tips from grass lines.

APPROACH: First we will initiate microbe free lines of each species and then develop a system of micropropagation to provide plant material for cryopreservation. This research is funded by the US Golf Association Turfgrass and Environmental Research Program. H.G. Hughes, Colorado State is responsible for the culture aspects and L.E. Towill for the cryopreservation aspects.

RESULTS: Plants free from microbes have been isolated from all four lines. These have been rather slow in growth and proliferation has only occurred in buffalograss and saltgrass. As an initial step in cryopreservation, alternative viability tests (tetrazolium salts, FDA) to regrowth are being examined from greenhouse grown materials. Preliminary tests suggest that tetrazolium should provide a semiquantitative estimate of bud survival and should allow use of buds from greenhouse plants to define some aspects of cryopreservation protocols until in vitro plant materials are available.

PRESERVATION OF SEEDS WITH UNKNOWN OR NON-ORTHODOX CHARACTERISTICS

JENNIFER CRANE (PI Physiol), C. Walters (PI Physiol), L. Hill (Biol Res Tech), R. Freeman (student), R. Krueger (ARS, Riverside, CA), F. Zee (ARS, Hilo, HI), J. Perscell (Leech Lake Tribal Council, MN)

PROBLEM: The physiology of seeds dictates the most appropriate storage strategy. Some species produce seeds that are non-orthodox, meaning that they do not survive desiccation and so can not be stored dry. Many tropical fruit crops, tree species, and plants growing in threatened habitats produce seeds of this category. The physiology of seeds from vegetatively-propagated crops, wild relatives of commercially produced crops, or plants growing in threatened habitats may be unknown and so appropriate storage conditions are also unknown. In order to preserve the genetic diversity of these valuable resources, the storage physiology of the seeds must be established and appropriate storage protocols determined. In addition, appropriate culture methods need to be established for recovery of these materials following storage.

APPROACH: The storage physiology of seeds is defined in terms of a mature seed's tolerance to freezing and desiccation stress. With this information, the appropriate storage procedure become apparent: seeds that appear orthodox can be stored using conventional procedures (optimizing water content and storing at -18C), and seeds that are non-orthodox must be cryopreserved in liquid nitrogen. Experiments consist of measuring the viability of seeds and excised embryonic axes following exposure to a range of moisture contents and low temperatures. Water sorption isotherms are used to predict the optimum water content for storage at subzero temperatures. Freezing characteristics of water in the seeds are measured using differential scanning calorimetry and provide insights into the feasibility of cryopreserving a species for which there is no published information.

Using rapid cooling procedures, embryonic axes of several species of non-orthodox seeds survive exposure to liquid nitrogen. Thus, cryopreservation protocols only require fine-tuning to maximize survival and increase handling efficiency. Efforts are now underway to monitor survival in liquid nitrogen in a pilot project using species of Rutaceae (citrus family), papaya, macadamia, and wild rice.

RESULTS: This research program officially began in Nov 1998. Survival of embryonic axes exposed to liquid nitrogen range from 70 to 100%. Next year's harvest of seeds will be used to initiate the pilot project. Curators of plants that produce seeds with non-orthodox or unknown physiologies are encouraged to contact Ms. Crane jcrane@lamar.colostate.edu) or Dr. Walters (chrisv@lamar.colostate.edu)

Ex Situ Conservation of the Endangered Texas Wild Rice *(Zizania texana)*

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PROBLEM: *Zizania texana is* a critically endangered wild rice species found only in a four km stretch of the upper San Marcos river in southern Texas. It is closely related to the more common and often cultivated wild rice species, *Zizania palustris,* and as such may have agronomic importance. Ex situ germplasm strategies should facilitate conservation of the genetic diversity of this species. However, *Zizania texana* does not often produce seeds in the wild, and the seeds that are produced cannot withstand desiccation to water contents that are amenable to conventional seed storage protocols. Furthermore, genetic assessment of extant populations of *Zizania texana will* aid in maximizing the genetic diversity of the ex situ collection.

APPROACH: This study has two components, the development of cryostorage procedures for *Zizania texana* and the assessment of genetic diversity of populations of the species. Because of the limited biodiversity of Z. *texana* and the difficulties in cryopreserving propagules, two strategies will be used to obtain an ex situ collection that is representative of the genetic diversity of plants growing in the wild. In one strategy, seeds, collected from plants in the wild (if there are sufficient supplies) or from plants grown in "captive" collections, will be cryopreserved. In the second strategy, clones of plants that give the greatest level of genetic diversity will be cryopreserved as shoot tips. At each step of the conservation effort, the genetic heterogeneity of the accession will be compared to that existing in wild populations to determine how much of the genetic diversity of the species can be preserved.

In order to reduce impact on *Zizania texana* populations, initial studies on the development of cryostorage procedures used the closely related common wild rice, *Zizania palustris,* with successful procedures then being tested for *Zizania texana.* The use of a combination of rapid drying, ultra rapid cooling, and tissue culture procedures were used to optimize cryostorage protocols.

Microsatellites were chosen to assess the genetic diversity of *Zizania texana. To* develop markers, a combination of screening genomic DNA with markers developed for closely related species and screening a genomic library for microsatellites is required. This should provide high number of polymorphic markers suitable for assessing genetic variability within a species.

RESULTS: Successful cryostorage was achieved for the cultivated wild rice, *Zizania palustris.* The optimal procedure (89% post-thaw survival) involved rapidly drying embryos to a water content of 0.3 g/g dry weight followed by cooling in a sub cooled liquid nitrogen (-208 C). This same procedure did not result in high survival of embryos of *Zizania texana (11 % post-thaw* survival). This suggested that, although Z. *texana* and *Z. palustris* are closely related, their seed physiology differs significantly in terms of their ability to survive cryostorage.

Microsatellite markers developed for *Oryza sativa,* the closest agronomically related species to *Zizania,* were applied to *Zizania texana* without success. Preliminary results suggest that the screening of a genomic library for microsatellites is a more reliable procedure for developing polymorphic markers for this species.

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Note: Names in caps are present or former ARS-NSSL employees

STATUS OF NPGS CROPS, June 1999

Species/Crop	Active site location	NSSL only no.	NPGS no.	NSSL no.	Backup at NSSL % 1999	Backup at NSSL % 1998	Backup at NSSL % 1997	Tested for germ %	Germ >64%	Seed no.> 549 %	Core %
Aegilops	Aberdeen		2182	1826	84	87	97				
Avena / oat	Aberdeen	628	21662	20604	95	96	94	65	99	21	4
Hordeum / barley	Aberdeen	732	27517	26890	98	97	97	72	99	63	8
Oryza / rice	Aberdeen	867	18034	17490	97	92	88	92	90	89	6
Secale / rye	Aberdeen	5	1910	1881	98	98	98	88	98	94	
Triticum / wheat	Aberdeen	1065	47137	46820	99	99	99	89	99	27	10
Aberdeen backup		**3308**	**120394**	**117825**	**98**	**97**	**96**				
Amaranthus	Ames		3397	2791	82	74	71	100	95	73	
Brassica / oilseed, vegetable	Ames	140	3328	3202	96	96	97	99	85	97	
Cucumis / cucumber, cantaloupe	Ames	482	5187	3618	70	57	48	68	94	63	
Cucumbers /pumpkin, squash	Ames	107	1114	730	66	64	52	78	98	75	
Daucus / carrot	Ames	170	962	764	79	86	87	93	54	94	
Helianthus / sunflower	Ames	187	3918	2722	69	66	59	88	91	84	3
Linum / flax	Ames	32	2992	2840	95	95	95	100	100	99	
Melilotus / sweetclover	Ames		909	739	81	79	78	96	89	96	9
Zea / corn	Ames	1494	18398	13475	73	71	74	86	95	79	1
Ames backup		**3066**	**50160**	**36362**	**73**	**73**	**70**				
Gossypium / cotton	College Station	1092	8155	4219	52	56	56	99	92	90	
Allium / onion	Geneva	29	1314	621	47	47	28	74	53	96	
Brassica oilseed, vegetable	Geneva	334	2572	1084	42	42	29	75	88	100	
Cucurbita / pumpkin, squash	Geneva	110	938	542	58	57	57	96	99	93	
Lycopersicon / tomato	Geneva	1556	7349	6635	90	90	70	91	88	99	
Geneva backup		**2375**	**14293**	**9931**	**70**	**73**	**56**				
Abelmoschus / okra	Griffin	40	3100	1953	63	63	57	35	87	35	5
Andropogon **	Griffin	1063	1098	1074	98	98	97	97	27	86	
Arachis / peanut	Griffin		9638	8132	84	1	67	68	83	56	8
Capsicum / pepper	Griffin	176	4057	2263	56	55	45	100	88	99	

Species/Crop	Active site location	NSSL only no.	NPGS no.	NSSL no.	Backup at NSSL % 1999	Backup at NSSL % 1998	Backup at NSSL % 1997	Tested for germ %	Germ >64%	Seed no. > 549%	Core %
Citrullus	Griffin	335	1947	1809	93	92	87	69	91	42	
Cucurbila /pumpkin, squash	Griffin	49	1293	833	64	61	19	17	89	15	
Cyamopsis / guar	Griffin	888	1300	1294	99+	99	99	93	90	92	
Eleusine / finger millet	Griffin	701	1473	1005	68	68	57	100	97	100	
Ipomoea / sweet potato seed	Griffin	35	481	200	42	44	11	21	86	8	
Pennisetum / pearl millet	Griffin	73	827	682	82	78	76	91	82	53	
Sesamum / sesame	Griffin	26	1229	1229	100	100	100	99	91	96	
Solanum eggplant	Griffin	2	939	909	97	94	96	96	80	97	12
Sorghum sorghum	Griffin	10829	40901	34583	85	79	65	99	79	97	6
Vigna / cowpea, mungbean	Griffin	275	12798	10177	80	70	46	59	97	58	9
Trifolium / clover	Griffin	34	2004	1454	73	73	24	78	95	77	5
Griffin backup		**16149**	**96334**	**74081**	**77**	**75**	**61**				
Trifolium / clover	Lexington		253	175	69	69	70	12	67	9	
Nicotiana / tobacco	Oxford	78	2159	1409	65	66	65	100	66	99	
Allium / onion	Pullman	30	677	171	25	20	21	99	84	100	
Beta / beet	Pullman	197	2391	1817	76	77	77	99	71	98	4
Bromus	Pullman	5	1109	981	88	89	86	99	84	99	
Carthamus / safflower	Pullman	25	2424	1957	81	80	80	99	80	99	9
Cicer / Chickpea	Pullman	9	4614	4029	87	87	85	98	100	96	11
Cucurbita / pumpkin, squash	Pullman	3	30	13	43	43	43	100	10	100	
Dactylis orchardgrass	Pullman	10	1532	1355	88	87	90	99	92	100	
Elymus wildrye, wheatgrass	Pullman	1	1821	1145	63	66	65	92	88	99	
Eragrostis	Pullman	1	1297	1273	98	98	98	99	91	100	
Festuca / fescue	Pullman	169	2279	1951	86	86	81	99	88	100	
Lactuca / lettuce	Pullman	243	1562	1247	80	80	80	99	68	99	
Lens / lentil	Pullman	3	2836	2435	86	84	84	100	100	100	10
Lolium / ryegrass	Pullman	147	1413	1096	78	77	79	99	95	99	2
Lotus / trefoil	Pullman	25	940	471	50	50	48	100	93	100	4
Medicago / alfalfa	Pullman	456	4128	3408	83	84	67	100	90	99	

Species/Crop	Active site location	NSSL only no.	NPGS no.	NSSL no.	Backup at NSSL % 1999	Backup at NSSL % 1998	Backup at NSSL % 1997	Tested for germ %	Germ >64%	Seed no. > 549%	Core %
Medicago / except alfalfa	Pullman	3	3513	3122	89	83	87	99	97	95	
Phaseolus / bean	Pullman	286	14119	10678	76	60	63	77	97	56	3
Pisum / pea	Pullman	548	3543	2992	84	74	74	99	99	96	14
Poa / bluegrass	Pullman	80	931	812	87	90	89	97	92	99	2
Trifolium / clover	Pullman	43	3762	2040	54	50	52	62	85	61	5
Pullman backup		**2449**	**70737**	**51858**	**73**	**73**	**72**				
Glycine / soybean	Urbana	1062	20657	17002	82	81	81	83	93	64	
Hordeum genetic stocks	Aberdeen GSHO		2531	1123	44	45	45				
Lycopersicon genetic stocks	Davis GSLY		3017	3017	100	100	100	46	97	50	
Pisum genetic stocks	Pullman GSPI		485	0	0	0					
Zea genetic stocks	Urbana GSZE		2537	2172	86	85	56	4	96	8	
Genetic Stocks Backup			**8570**	**6312**	**74**	**73**	**62**				
Fragaria / strawberry seed	Corvallis	5	5	5	100	100	100	79	45	35	
Malus / apple seeds	Geneva		1416	489	35	0					
Solanum / potato seed	Sturgeon Bay	9	4541	3975	88	88	90	94	96	88	54
Seeds of Vegetative Backup			**5962**	**4469**	**75**	**74**					
TOTAL SEED BACKUP		**30081**	**400095**	**325238**	**81**	**78**					
VEGETATIVE											
Corylus filbert, hazelnut	Corvallis		637	3				100	67		26
Cydonia quince	Corvallis		90	1	1	1					51
Fragaria strawberry	Corvallis		1549	0	0	0					33
Humulus hop	Corvallis		984	12pollen	1	0					8
Juglans walnut	Corvallis		18	0	0	0					78
Mentha mint	Corvallis		514	36	7	2		100	3		10
Pycnanthemum / mountain mint	Corvallis		110	0	0	0					27
Pyrus / pear	Corvallis		1927	71	4	3		100	25		12
Ribes / currant, gooseberry.	Corvallis		1070	3	0	0		100	100	100	30

Species/Crop	Active site location	NSSL only no.	NPGS no.	NSSL no.	Backup at NSSL % 1999	Backup at NSSL % 1998	Backup at NSSL % 1997	Tested for germ %	Germ >64%	Seed no. > 549%	Core %
Rubus / raspberry, blackberry	Corvallis		1767	234	13	0		1	100	67	30
Vaccinium / blueberry, cranberry	Corvallis		1276	0	0	0					29
Juglans / walnut	Davis		349	0	0	0					
Prunus	Davis		1044	0	0	0					
Vitis / grape	Davis		2421	0	0	0					
Malus / apple			3093	1820	59	50		74	63		
Prunus	Geneva		81	19	23	0		100	42		
Vitis / grape	Geneva		1228	0	0	0					
Ipomoea / sweet potato clonal	Griffin		623	38	6	6		74			
Prunus	National Arboretum		251	0	0	0					
Allium / garlic	Pullman		204	0	0	0					
Citrus	Riverside		886	0	0	0					
Solanurn / potato clonal	Sturgeon Bay		728	0	0	0					98
VEGETATIVE BACKUP			**31833**	**1908**	**6**	**5**					
TOTAL ACCESSIONS	**NPGS**	**30081**	**431928**	**327236**	**76**	**73**					

**Species with limited longevity at 5 C

Appendix 9

Plant Genetic Resources Conservation Unit
Administration

Personnel

Dr. Robert Lynch, Acting Research Leader
Rella Harrison, Administrative Secretary (S-9)
Marie Gimbrone, Secretary (ARS)
Carolyn Toney, Athens Location Support Office

PGRCU
USDA-ARS
CRIS Allocations

CRIS Number	Title	Amount
6607-21000-007-00D	Crop Conservation and Genetic Analysis	$1,344,633
6606-22000-004-00D	Diagnosis and Elimination of Pathogens in Introduced Plant Germplasm	$150,794
		$1,495,427

PGRCU
S-9 Allocations

Source	Amount
State Agric. Experiment Station Directors (S-9)	**$324,357**

PGRCU
Total Funding

Agricultural Research Service	$1,495,427
State Agric. Experiment Station Directors (S-9)	$324,357
Total Allocations	$1,819,784

PGRCU
Expenses

Base Funding	**$1,819,784**
Indirect Research Costs	
Administrative Office	200,091
Repair and Maintenance	29,900
RSA Janitorial/Field Services	50,000
Mandated transfer of funds to Tifton	29,400
Balance	**$1,501,393**
Personnel	
Federal	$1,030,650
Research Support Agreement	82,354
S-9	281,930
Balance	**$115,716**
Other Operating Expenses	
Vehicle Insurance	$5,676
Balance	$100,040

Balance = Operating Funds	$100,040
Curator Operating Funds ($10 – 23K)	$ 78,000
Research Leader Operating Funds (Molecular Genetics Lab, Farm Services Computer Specialist, Germplasm Storage/ Distrib. Unit)	$ 32,040

1999 Operating

Lapse Salaries:

Research Coordinator II (S. Mitchell)	$59,568
Research Coordinator II (M. Newman)	$30,000

Purchases:
2 vehicles

Projected Expenses – FY 2000

	1998 – 1999	1999 – 2000
Balance	**$1,510,393**	**$1,510,393**
Personnel		4% Increase
Federal	$1,030,650	$1,071,876
Research Support Agreement	87,112	90,596
S-9	281,930	293,207
Balance	**$110,701**	**$54,714**

Appendix 10

AN INFORMATION UPDATE FROM ***June, 1999***

Cooperative State Research, Education, & Extension Service
Plant and Animal Systems

FY 2000 Agriculture Appropriations

On Thursday, May 13, the House Agriculture Appropriations Committee Marked-Up the FY 2000 Agriculture budget on Wednesday, May 19, 1999. The bill, which is yet to be approved by the House, provides $920.9 million for CSREES, which is $2.9 million below the FY 1999 level of $923.8 million, and $27.1 million below the FY 2000 budget request of $948 million.

As a result of the House Agriculture Appropriations Mark-Up, most of the Agency's programs remained at their FY 1999 levels, including the formula programs and other research, extension, and educational programs. The exceptions were an increase of $1.0 million in Extension Federal Administration, $10.0 million for unspecified activities under the Integrated Activities Account, and a decrease in the National Research Initiative (NRI) from $119.3 million in FY 1999 to S105.4 million in FY 2000, a reduction of $13.9 million.

As in FY 1999, a General Provision blocked the use of funds to carry out the Fund for Rural America and the Initiative for Future Agriculture and Food Systems. Another General Provision raised the cap on indirect cons for research grants from 14 percent to 19 percent, and continued to exempt the Small Business Innovation Research Program from this limitation.

On Tuesday, May 25, House Floor Action on the Agricultural Appropriations Bill began with over 100 amendments being introduced designed to decrease funding for USDA., including CSREES. These amendments, which were part of a larger controversy concerning spending caps and the social security surplus, resulted in debates so intense that on May 27, the House managers withdrew the bill from the consideration. Although, they plan to resume debate on Tuesday, June 8th, it appears that there has been no change in positions over the Memorial Day Recess and the amendments to decrease funding will be considered

It is anticipated that the Senate will mark-up the Agriculture Appropriations Bill sometime in June. For additional details on CSREES budget -www.reeusda.gov/budget/webfund.htm

Revisions to CRIS system

Codes in the CRIS system are used by federal managers to track spending in particular areas. The recent revision is to update the coding to reflect new fields of study and revise or delete existing codes. The general intent was to focus on problem areas, but some research areas are basic or are separated out for necessary reporting requirements. In the area of plant breeding, the categories can be roughly divided into basic and applied research (see table below). Additionally, they reflect new areas of emphasis in genome and germplasm. RPAs for basic research are:

- 201 for the basics of plant breeding, including techniques, molecular tools, sequencing, transformation, inheritance of traits, and genome databases;

- 202 for discovery, acquisition, preservation, characterization and development of germplasm, including evaluation even if searching collection for a specific trait; and
- 206 -for research on metabolic pathways, including those related to seed development respiration, and germination.
- RPAs 203, 204, 211, 212, 213 will encompass more applied breeding. Any breeding for a specific characteristic with the intent of releasing a breeding line or cultivar. Variety testing probably is most appropriately assigned to RPA 203 (yield). If you think your work encompasses more than one RPA, it probably does. Please use percentages and divide it up.

In addition to RPAs, changes to other codes include:

- Field of Science- 1080 Genetics, including Plant Breeding
- Commodity codes - All have changed and sub-commodity codes were eliminated
- Activity code - no longer used

Primary RPAs for plant breeding, genetics and germplasm

201	Plant Genome, Genetics, and Genetic Mechanisms
202	Plant Genetic Resources and Biodiversity
203	Plant Biological Efficiency and Abiotic Stresses Affecting Plants
204	Plant Product Quality and Utility
206	Basic Plant Biology
211	Insect, Mites and Other Arthropods Affecting Plants
212	Diseases and Nematodes Affecting Plants
213	Weeds Affecting Plants

Personnel Changes

New Administrator Dr. Charles Laughlin will be the new Administrator for CSREES. Since July 1996, Dr. Laughlin has been the Dean and Director of the College of Tropical Agriculture and Human Resources at the University of Hawaii-Manoa. He also held administrative positions at Colorado State, Univ. of Georgia, Mississippi State, Michigan State, and Univ. of Florida. Dr. Laughlin received a B.S. degree from Iowa State University, an M.S. degree from Univ. of Maryland, and a Ph.D. from VPISU with a major in plant pathology and physiology.

New National Program Leader for Horticulture: Plant and Animal System has completed the interview process for a new National Program Leader for Horticulture. We expect to complete the selection process by June 30.

Other news and information, including current RFPs and personnel changes can be on our website: www.reeusda.gov

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United States Department of Agriculture **Agricultural Research Service** **Southern Plains Area**
P.O. Box 159, Hwy. 3 West
Lane, Oklahoma 74566

June 2, 1999

SUBJECT: Status of *Citrullus* Germplasm Collection – Final Report

TO: Watermelon Research and Development Working Group

FROM: Benny Bruton, Chairman
U.S. Department of Agriculture
Agricultural Research Service
Lane, Oklahoma 74555

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The purpose of this memorandum is to provide specific information to you on the "*Citrullus* Germplasm Collection" located at Griffin, Georgia. Over the years, most of you have heard that there is need for a major effort to get the collection into good shape. Dr. Bob Jarret, Curator at the Plant Genetic Resources Unit at Griffin, was in California at the Cucurbit Crop Germplasm Committee Meeting in conjunction with the Cucurbitaceae'98 meetings. He provided a very informative lecture on watermelon germplasm at the Plant Genetic Resources Unit. It is my intent to provide some additional information that may be useful in further establishing the status of the Plant Introduction watermelon (PI) collection. I have called several people from seed companies and public research to determine if they have been able to acquire the PIs they requested. For the most part, their response has been that they received those requested. Dr. Jarret confirmed that there were a number of requests made last year for the entire *Citrullus* collection, but all requests could not be fully met. However, this is not uncommon for any germplasm collection.

There are a total of 1,644 *Citrullus* PIs with 1,393 (85%) available for distribution. This would seem to indicate that the collection is "nearly" in good shape. However, this is not the case! Of the 1,393 available, 121 accessions are down to an on-hand quantity of < 400 seeds. They are considered available, but inventories are below the critical replenishment level of 400 seeds. This does not take into consideration the likelihood that some seed lots may have low viability. Up until 1994, the *Citrullus* seed increases were open-pollinated. Consequently, the genes are still present**,** but it may be difficult to determine their original source. At present, there are approximately 100 cages for use at the Griffin Station with all increases being done in cages. Actually, *Citrullus* seed are presently increased at the ARS facility at Byron, Georgia (30 mi. south of Griffin). In 1998, approximately 70 PIs were planted for increase with approximately 20 lost due to soilborne disease. In the last 5 years, the increase of approximately 75 PIs per year have been attempted. Actual seed increases averaged only about 50 per year because of germination problems, plants didn't flower, or disease. The bottom-line is that there is some progress being made but at a less than desirable pace. Seeds are being stored at 20 C at Griffin. Under those conditions, the watermelon germplasm should last for 30 to 40 years, maybe more.

The Citrullus heirloom varieties (more than 300 cultivars) are stored at Ft. Collins in the National Seed Storage Laboratory (NSSL). Many of the varieties have been identified as "at risk" because of low seed number with unknown seed germination percentage as well as others with known low seed germination. The heirloom varieties at NSSL are not officially included in the inventory at Griffin. Information on the heirloom varieties is scant and therefore little data is available in Genetics Resource Information Network (GRIN). The heirloom varieties are also the responsibility of the Griffin Station to increase. However, the heirloom varieties are not being regenerated at this time. There are no facilities for regeneration at Ft. Collins. The heirloom varieties should be given a PI number and duplicate samples placed at Griffin, provided that the rate of regeneration at Griffin can be increased. At the rate of 50 PI increases per year, it will take about 14 years to regenerate the seed stocks that are presently in low volume at Griffin and the heirloom varieties stored at Ft. Collins. We need to be achieving about 100 to 150 PI increases per year to adequately maintain the germplasm.

Dr. Jarret was contacted some years ago by Dr, Bernard DeWinter (Botanist) from South Africa. Dr. DeWinter had a "very complete" collection of watermelon germplasm native to South Africa (the center of origin and diversity for *Citrullus lanatas*). Dr. DeWinter was seeking assistance to regenerate his collection and offered samples of all accessions in return for that assistance, At our January meeting in Memphis, Tennessee, the Watermelon Research and Development Working Group demonstrated an interest in acquisition of the germplasm. It should be a high priority to procure this collection because it should contain some very valuable genetic material. Dr. Robinson donated two accessions of *CitrulIus ecirrhosus* to the collection in 1999. Additional accessions of this species and accessions of *Citrullus rehmii* are needed. The current back-log of material awaiting regeneration may be negatively impacting efforts to take advantage of opportunities to introduce new and potentially valuable germplasm.

At present, data in the GRIN system is very incomplete, especially for disease resistance characteristics. If we want to find information on Fusarium wilt resistance, can we get it from the GRIN? Maybe! If you want PIs with multiple disease resistance, it may be impossible to get that information from the existing dbase. The GRIN system may never be complete for all desired characteristics. There is no back-log of data entry at Griffin. According to Dr, Jarret, he is not aware of any *Citrullus* evaluation data that has been submitted to Griffin for inclusion into GRIN in the last 5 years. This could mean that no PI screens were done during that time, that the data is not being properly submitted, or not being submitted through the correct channels, They do not compile data from published Journal reports. If more information was available in the GRIN system and data easier to access, there would surely be more requests for watermelon germplasm than present. According to the Cucurbit Crop Germplasm Committee Report- 1996, there were 2,459 seed samples distributed from 1980 through 1988 (1,155 domestic requests and 1,304 international requests). This may be a relatively small number of requests but no reflection of the importance of the germplasm collection.

What is the risk to the watermelon industry if improvements are not made in the increase and maintenance of the watermelon germplasm collection? Our PI collection may be our only source for resistance genes to diseases and insect pests that emerge or are introduced in the future. This is especially true when adequate resistance is not already available in commercial cultivars. The Cucurbit Crop Germplasm Committee-1996 has classified what they consider to be critical areas for watermelon germplasm evaluation. Of the ten priority areas targeted for germplasm evaluation, seven were for disease or insect pests. In the last ten to fifteen years, we have experienced three watermelon diseases new to the United States that illustrate the importance of our germplasm collection. Each of the three diseases are potentially devastating to watermelon production. One thing is for sure. We can expect there will be additional new diseases to contend with in the future. The three new diseases mentioned above include:

1. Bacterial Fruit Blotch. Watermelon fruit blotch, caused by a seedborne bacterium, was first observed commercial production fields in 1989. The disease has been found in several watermelon production states, primarily in the eastern U.S. Fruit of some cultivars are more susceptible to bacterial fruit blotch than others which appears to be related to fruit color. The most susceptible fruit are those with a light green rind such as 'Charleston Gray'. Cultivars with a light and dark green stripe, such as 'Crimson Sweet', are more tolerant and the most tolerant are those with a solid dark-green color such as 'Sugar Baby'. However, the level of resistance in commercial cultivars is not sufficient when conditions favor fruit blotch development.

***2. Fusarium oxysporum* f. sp. *niveum* race 2.** Race 2 of the watermelon Fusarium wilt fungus was first reported in Texas in 1985. The fungus has since been found in Oklahoma and Florida. At present, race 2 is not widespread within watermelon production states, although the potential for further spread to other watermelon producing areas is great because the disease can be seedborne. There are no commercially acceptable hybrids or cultivars with resistance to the highly virulent rare 2. Resistance has been reported in the PI-296341-FR. Consequently, PIs maybe our only source of resistance to race 2.

3. Yellow Vine. Yellow vine is a relatively new disease of watermelon, caused by a phloem-limited bacterium. Evidence indicates that insects (leafhopper) vector the disease. The disease was first observed in central Texas and Oklahoma in 1991 and has caused severe losses in early-planted watermelons in some years. In 1998, the disease was detected in watermelon and pumpkin in Tennessee. Consequently, production areas of Georgia, Florida, and other parts of the southeast U S may be at risk in the future.

Presently, low levels of resistance or tolerance have been identified in a few open-pollinated and hybrid cultivars, although the mechanism of resistance is unknown. PIs will be evaluated in the future as a source of resistance genes to the yellow vine disease.

Specific recommendations will not be submitted because we are not familiar with the organizational structure. However, the Cucurbit Crop Germplasm Committee filed a report in 1996 with additional information and specific recommendations. For a copy of that report, you can contact Dr. Alan Stoner, USDA-REE-ARS-BA-PSI-NGR LAB, BLDG 003 BARC-WEST RM 101, 10300 Baltimore Blvd, Beltsville, MD 20705-2350. His e-mail address is: astoner@ars-grin.gov. In addition, Dr. Peter K. Bretting (USDA-ARS) is the National Program Staff (NPS) responsible for plant germplasm. Dr. Bretting is concerned about the status of the watermelon germplasm collection and wants to work with the Watermelon Research and Development Working Group and any other interested parties to improve the condition of the collection. If you have questions, he can be contacted at (301) 504-5541. His e-mail address is: pkb@ars.usda.gov.

cc:
American Seed Trade Association
G. Arkin
W. Blackburn
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R. Breeze
Cucurbit Crop Germplasm Committee
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National Watermelon Association
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